PA NT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 August 2000 (25.08.00)	
International application No. PCT/US99/31284	**Applicant's or agent's file reference** P23,565-A PC
International filing date (day/month/year) 30 December 1999 (30.12.99)	**Priority date** (day/month/year) 30 December 1998 (30.12.98)
Applicant BRUNO, René	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 July 2000 (27.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Philippe Bécamel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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Aventis
23565-A PCT
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SYNNESTVEDT & LECHNER
TEN: PJK

PATENT COOPERATION TREA

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

ENTERED COMPUTER
8-29-00

To:
Synnestvedt & Lechner LLP
2600 Aramark Tower
Attn. Kelly,Patrick,J.
1101 Market Street
Philadelphia,PA 19107-2950
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)*	29/06/2000
Applicant's or agent's file reference: P23,565-A PC	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/US 99/ 31284	International filing date *(day/month/year)*: 30/12/1999
Applicant: Aventis Pharmaceuticals Products Inc et al	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed Instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Jaap Hurenkamp

Form PCT/ISA/220 (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P23,565-A PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 31284	30/12/1999	30/12/1998
Applicant		
Aventis Pharmaceuticals Products Inc et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

Method for determining the dosage of a taxoid to administer to a cancer patient, and for assessing the effects and reducing the side effects of taxoid treatment on a cancer patient, based on the alpha-1-acid glycoprotein levels of that patient compared to the average alpha-1-acid glycoprotein levels in a population of patients suffering from that cancer and being treated with that taxoid.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]S 99/31284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	VEYRAT-FOLLET C. ET AL.: "Application of clinical trial simulation in exploring the safety profile of docetaxel (D) in cancer patients" CLINICAL PHARMACOLOGY AND THERAPEUTICS, vol. 65, no. 2, February 1999 (1999-02), page 198 XP000908903 abstract --- URIEN S. ET AL.: "Docetaxel serum protein binding with high affinity to alphal-acid glycoprotein" INVESTIGATIONAL NEW DRUGS, vol. 14, 1996, pages 147-151, XP000908900 abstract page 150, column 1, paragraph 2 -page 151, column 1, paragraph 1 --- -/--	1-13 1-13

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2000	29/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Pellegrini, P

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BRUNO R. ET AL.: "A population pharmacokinetic model for docetaxel (Taxotere): model building and correlation" JOURNAL OF PHARMACOKINETICS AND BIOPHARMACEUTICS, vol. 24, no. 2, - 1996 pages 153-172, XP000908881 cited in the application abstract page 169, paragraph 5 page 170, paragraph 5 ---	1-13
X	BRUNO R. ET AL.: "Population pharmacokintetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer" J. CLIN. ONCOL., vol. 16, no. 1, January 1998 (1998-01), pages 187-196, XP000910385 cited in the application abstract page 191, column 1, line 18-20 ---	1-13
X	GANZ P.A. ET AL.: "Monitoring the therapy of lung cancer with alpha-1-acid glycoprotein" CANCER RESEARCH, vol. 44, 1984, pages 5415-5421, XP000909045 abstract page 5416, column 1, paragraph 3 -page 5416, column 2, paragraph 1 ---	1-13
X	GANZ P.A. ET AL.: "Evaluation of a radioimmunoassay for alpha-1-acid glycoprotein to monitor therapy of cancer patients" JOURNAL NATIONAL CANCER INSTITUTE (JNCI), vol. 71, no. 1, 1983, pages 25-30, XP000909046 cited in the application abstract page 26, column 1, paragraph 2 ---	1-13
X	BIENVENU J. ET AL.: "Laser nephelometry of orosomucoid in serum of newborns: reference intervals and relation to bacterial infections" CLIN. CHEM., vol. 27, no. 5, 1981, pages 721-726, XP002139731 cited in the application page 721, column 2, paragraph 5 -page 722, column 1, paragraph 2 ---	1-13

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KREMMER T. ET AL.: "Determination and analysis of human serum alpha-1-acid glycoprotein by liquid chromatographic methods" J. LIQUID CHROMATOGRAPHY, vol. 18, no. 6, 1995, pages 1207-1218, XP000909051 page 1209, paragraph 1 -page 1210, paragraph 2 -----	1-13

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 14-29

Subject-matter for which protection os sought in claims 14-29 is excluded from patentability because it relates to a method for treatment of the human or animal body by therapy (Rule 39.1.(iv) PCT). However, a search has been performed on analytical tests for determining alpha-1-acid glycoprotein levels and on the correlation between alpha-1-acid glycoprotein levels and the pharmacokinetics/pharmacodynamics of taxoids.

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 99/31284

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 14-29
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

NOTE FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the International application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

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PATENT COOPERATION TREATY

Aventis
23565-A PCT
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APR 24 2001
SYNNESTVEDT & LECHNER
ATTEN: PJK

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Kelly,Patrick,J.
Synnestvedt & Lechner LLP
2600 Aramark Tower
1101 Market Street
Philadelphia,PA 19107-2950
ETATS-UNIS D'AMERIQUE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	12.04.2001
Applicant's or agent's file reference P23,565-A PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/31284	30/12/1999	30/12/1998

Applicant
Aventis Pharmaceuticals Products Inc. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Digiusto, M Tel.+49 89 2399-8162

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P23,565-A PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/31284	30/12/1999	30/12/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant Aventis Pharmaceuticals Products Inc. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/07/2000	12.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Jacques, P Telephone No. +49 89 2399 8934

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/31284

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

2-20,22-47	as originally filed	
1,21	with telefax of	22/03/2001

Claims, No.:

1-29	as originally filed

Drawings, sheets:

1/4-4/4	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 14-29 (with respect to industrial applicability).

because:

   ☒ the said international application, or the said claims Nos. 14-29 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 14-29 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
   In this context, the said claims are considered to fall under the concept of methods of treatment (see further point 13 under Item V).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

   D1: BRUNO R. ET AL.: 'Population pharmacokintetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer' J. CLIN. ONCOL., vol. 16, no. 1, January 1998 (1998-01), pages 187-196.

2. All the other documents cited as X-documents in the I.S.R. have not been considered as pertinent as all of the said documents fail to disclose any relationship between the level of alpha-1-acid glycoprotein (AAG) in patients having been treated with a taxoid for cancer and the clinical outcome thereof

3. The priority documents of the present application were not available at the time that this report was written. Consequently, the document cited as P'X' in the I.S.R. may become relevant to the question of novelty of some or all of the claims at a later stage of the procedure.

4. As the particular combination of features of independent claim 1 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

5. However, the subject-matter of the said claim does not involve an inventive step in the sense of Article 33(3) PCT for the following reasons:
The closest state of the art is considered to result from document D1.
This document discloses that a high AAG level is predictive of poor prognosis in response to both NSCLC and breast cancer: namely decreasing the chances of response and increasing the risk of relapse (see page 194, lines 2 to 5). Moreover, the said document discloses the AAG level as being the only significant predictors for febrile neutropenia, a side effect of patient being treated with docetaxel; the higher the AAG level, the lower the odds of experiencing grade 4 neutropenia during the first course of treatment (see page 191, left column, line 5 to right column, line 5).
Thus, the said document clearly discloses that for cancer patients being treated with Docetaxel (a taxoid), the patient's AAG level is a prognostic factor for the clinical outcome.

The subject-matter of claim 1 is distinguished therefrom in that the level of AAG from a patient being treated for cancer with a taxoid is evaluated (steps A and B) and compared to a predetermined AAG level derived from a population of patients having said cancer and treated with said taxoid at a common dosage.
The technical effect of this distinguishing feature results in determining the dosage of a taxoid to administer to a patient who is being treated for cancer.
The technical problem to be solved by the invention was therefore to provide a method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer.

It is clear from D1 that the level of AAG is a prognostic factor for clinical outcome. More precisely, D1 discloses the relationship between AAG level and the response to treatment, survival and side effects for a patient population having cancer and being treated with a taxoid.
Thus, it would fall within the normal design capabilities of the skilled man to evaluate and compare the level of AAG of a patient having cancer, and being treated with a taxoid, to a predetermined AAG level derived from a population of patients having the same cancer and having been treated with the same taxoid, to consider recommending an adjustment in the taxoid dosage.
Therefore, the subject-matter of claim 1 does not involve an inventive step in the sense of Article 33(3) PCT.

6. Dependent claims 2 to 5 do not appear to contain any additional features which meet the requirements of inventive step as all the features of these claims are conventional in the art.

7. As the particular combination of features of independent claim 6 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

8. However, the subject-matter of the said claim does not involve an inventive step in the sense of Article 33(3) PCT as D1 already discloses the relationship between the level of AAG and response to treatment, survival and side effects for a patient population having cancer and being treated with a taxoid (see reasoning under point 5 above).
Thus, the same reasoning as for claim 1 applies to claim 6 and consequently it does not involve an inventive step in the sense of Article 33(3) PCT.

9. Moreover, dependent claims 7 to 13 do not appear to contain any additional features which meet the requirements of inventive steps as all the features of these claims are conventional in the art.

10. Notwithstanding the objection raised under Article 34(4)(a)(i) PCT (see point 1 under Item III), as the particular combination of features of independent claim 14 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

11. However, the subject-matter of the said claim does not involve an inventive step in the sense of Article 33(3) PCT as document D1 already discloses the relationship between the level of AAG and side effects for a patient population having cancer and being treated with a taxoid (see further discussion under point 5 above).
Thus, the same reasoning as for claims 1 and 6 applies to claim 14 and consequently it too does not involve an inventive step in the sense of Article 33(3) PCT.

12. The same comments apply to dependent claims 15 to 29 which do not contain any additional features which meet the requirement of inventive step as all the said features are conventional in the art or fall within the normal design capabilities of the

skilled man (Article 33(3) PCT).

13. For the assessment of the present claims 14-29 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

1. References to U.S. application serial numbers are present in the description (see page 21, lines 11-12).

14 Jan

PATENT COOPERATION TREATY

PCT

REC'D 19 APR 2001

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P23,565-A PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/31284	30/12/1999	30/12/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant Aventis Pharmaceuticals Products Inc. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/07/2000	12.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Jacques, P Telephone No. +49 89 2399 8934

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/31284

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

2-20,22-47	as originally filed	
1,21	with telefax of	22/03/2001

Claims, No.:

1-29	as originally filed

Drawings, sheets:

1/4-4/4	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 14-29 (with respect to industrial applicability).

because:

   ☒ the said international application, or the said claims Nos. 14-29 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 14-29 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
   In this context, the said claims are considered to fall under the concept of methods of treatment (see further point 13 under Item V).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

   D1: BRUNO R. ET AL.: 'Population pharmacokintetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer' J. CLIN. ONCOL., vol. 16, no. 1, January 1998 (1998-01), pages 187-196.

2. All the other documents cited as X-documents in the I.S.R. have not been considered as pertinent as all of the said documents fail to disclose any relationship between the level of alpha-1-acid glycoprotein (AAG) in patients having been treated with a taxoid for cancer and the clinical outcome thereof

3. The priority documents of the present application were not available at the time that this report was written. Consequently, the document cited as P'X' in the I.S.R. may become relevant to the question of novelty of some or all of the claims at a later stage of the procedure.

4. As the particular combination of features of independent claim 1 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

5. However, the subject-matter of the said claim does not involve an inventive step in the sense of Article 33(3) PCT for the following reasons:
The closest state of the art is considered to result from document D1.
This document discloses that a high AAG level is predictive of poor prognosis in response to both NSCLC and breast cancer: namely decreasing the chances of response and increasing the risk of relapse (see page 194, lines 2 to 5). Moreover, the said document discloses the AAG level as being the only significant predictors for febrile neutropenia, a side effect of patient being treated with docetaxel; the higher the AAG level, the lower the odds of experiencing grade 4 neutropenia during the first course of treatment (see page 191, left column, line 5 to right column, line 5).
Thus, the said document clearly discloses that for cancer patients being treated with Docetaxel (a taxoid), the patient's AAG level is a prognostic factor for the clinical outcome.

The subject-matter of claim 1 is distinguished therefrom in that the level of AAG from a patient being treated for cancer with a taxoid is evaluated (steps A and B) and compared to a predetermined AAG level derived from a population of patients having said cancer and treated with said taxoid at a common dosage.
The technical effect of this distinguishing feature results in determining the dosage of a taxoid to administer to a patient who is being treated for cancer.
The technical problem to be solved by the invention was therefore to provide a method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer.

It is clear from D1 that the level of AAG is a prognostic factor for clinical outcome. More precisely, D1 discloses the relationship between AAG level and the response to treatment, survival and side effects for a patient population having cancer and being treated with a taxoid.
Thus, it would fall within the normal design capabilities of the skilled man to evaluate and compare the level of AAG of a patient having cancer, and being treated with a taxoid, to a predetermined AAG level derived from a population of patients having the same cancer and having been treated with the same taxoid, to consider recommending an adjustment in the taxoid dosage.
Therefore, the subject-matter of claim 1 does not involve an inventive step in the sense of Article 33(3) PCT.

6. Dependent claims 2 to 5 do not appear to contain any additional features which meet the requirements of inventive step as all the features of these claims are conventional in the art.

7. As the particular combination of features of independent claim 6 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

8. However, the subject-matter of the said claim does not involve an inventive step in the sense of Article 33(3) PCT as D1 already discloses the relationship between the level of AAG and response to treatment, survival and side effects for a patient population having cancer and being treated with a taxoid (see reasoning under point 5 above).
Thus, the same reasoning as for claim 1 applies to claim 6 and consequently it does not involve an inventive step in the sense of Article 33(3) PCT.

9. Moreover, dependent claims 7 to 13 do not appear to contain any additional features which meet the requirements of inventive steps as all the features of these claims are conventional in the art.

10. Notwithstanding the objection raised under Article 34(4)(a)(i) PCT (see point 1 under Item III), as the particular combination of features of independent claim 14 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

11. However, the subject-matter of the said claim does not involve an inventive step in the sense of Article 33(3) PCT as document D1 already discloses the relationship between the level of AAG and side effects for a patient population having cancer and being treated with a taxoid (see further discussion under point 5 above).
Thus, the same reasoning as for claims 1 and 6 applies to claim 14 and consequently it too does not involve an inventive step in the sense of Article 33(3) PCT.

12. The same comments apply to dependent claims 15 to 29 which do not contain any additional features which meet the requirement of inventive step as all the said features are conventional in the art or fall within the normal design capabilities of the

skilled man (Article 33(3) PCT).

13. For the assessment of the present claims 14-29 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

1. References to U.S. application serial numbers are present in the description (see page 21, lines 11-12).

Docket No. P23,565-A PCT

PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS

Field of the Invention

This invention relates to methods which are useful in the treatment of cancer with a pharmaceutical that is a member of the family of anti-neoplastic agents known as taxoids.

The family of anti-neoplastic agents known as taxoids are based on natural and modified compounds that have the taxone skeleton isolated from the yew tree (Taxaceae). Two particularly effective taxoids are paclitaxel, which is a natural product isolated from the Pacific yew (Taxus brevifolia), and docetaxel, which is a semisynthetic product derived from the needles of the European yew (Taxus baccata). The activities of these agents have been demonstrated in a wide variety of cancers, including breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. Other taxoids are being developed for cancer treatment also.

Examples

The following examples are representative of the practice of the invention.

Example 1

This example is illustrative of the present invention. It provides information stemming from a study of cancer patients who were treated with a taxoid (docetaxel) and concerning the relationship between AAG levels and a variety of physiological effects, including, for example, side effects. This study in its entirety is reported in applicant's U.S. provisional patent Application No. 60/114,520, filed December 30, 1998. The results of this study were published in Bruno et al., *Journal of Clinical Oncology*, Vol. 16, No. 1, p. 187-196 (1998).

The Patient Pool

Data were prospectively collected from patients entered in twenty-four Phase II open, non-randomized studies conducted from May 1992 to March 1994 to assess docetaxel clinical efficacy in a variety of tumor types including breast cancer, non small cell lung cancer, ovarian cancer, head and neck cancer, melanoma, renal cancer, colorectal cancer, gastric cancer, small cell lung cancer, and soft-tissue sarcoma. The studies were conducted in over 50 centers in Europe and three centers in the United States.

Criteria for eligibility included histology, at least one bidimensionally measurable lesion, adequate bone marrow reserve (absolute neutrophil count > 2,000/μL), adequate renal function (normal creatinine level) and liver function (total bilirubin level < 1.25 x ULN, SGOT (ALT) ≤2 x ULN or ≤3 x ULN in case of proven liver metastases). According to the tumor type, patients could have received various extents of prior treatment. The starting dose of docetaxel was either 75 mg/m² or 100 mg/m² given as a 1-hr infusion every 3 weeks. Dose reduction (25 %) or delay of subsequent courses were permitted, based on the degree of toxicity observed.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
P23,565-A PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 31284	30/12/1999	30/12/1998
Applicant		
Aventis Pharmaceuticals Products Inc et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 14-29

Subject-matter for which protection os sought in claims 14-29 is excluded from patentability because it relates to a method for treatment of the human or animal body by therapy (Rule 39.1.(iv) PCT). However, a search has been performed on analytical tests for determining alpha-1-acid glycoprotein levels and on the correlation between alpha-1-acid glycoprotein levels and the pharmacokinetics/pharmacodynamics of taxoids.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

Method for determining the dosage of a taxoid to administer to a cancer patient, and for assessing the effects and reducing the side effects of taxoid treatment on a cancer patient, based on the alpha-1-acid glycoprotein levels of that patient compared to the average alpha-1-acid glycoprotein levels in a population of patients suffering from that cancer and being treated with that taxoid.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]S 99/31284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	VEYRAT-FOLLET C. ET AL.: "Application of clinical trial simulation in exploring the safety profile of docetaxel (D) in cancer patients" CLINICAL PHARMACOLOGY AND THERAPEUTICS, vol. 65, no. 2, February 1999 (1999-02), page 198 XP000908903 abstract ---	1-13
X	URIEN S. ET AL.: "Docetaxel serum protein binding with high affinity to alphal-acid glycoprotein" INVESTIGATIONAL NEW DRUGS, vol. 14, 1996, pages 147-151, XP000908900 abstract page 150, column 1, paragraph 2 -page 151, column 1, paragraph 1 ---	1-13
	-/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2000	29/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pellegrini, P

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BRUNO R. ET AL.: "A population pharmacokinetic model for docetaxel (Taxotere): model building and correlation" JOURNAL OF PHARMACOKINETICS AND BIOPHARMACEUTICS, vol. 24, no. 2, - 1996 pages 153-172, XP000908881 cited in the application abstract page 169, paragraph 5 page 170, paragraph 5 ---	1-13
X	BRUNO R. ET AL.: "Population pharmacokintetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer" J. CLIN. ONCOL., vol. 16, no. 1, January 1998 (1998-01), pages 187-196, XP000910385 cited in the application abstract page 191, column 1, line 18-20 ---	1-13
X	GANZ P.A. ET AL.: "Monitoring the therapy of lung cancer with alpha-1-acid glycoprotein" CANCER RESEARCH, vol. 44, 1984, pages 5415-5421, XP000909045 abstract page 5416, column 1, paragraph 3 -page 5416, column 2, paragraph 1 ---	1-13
X	GANZ P.A. ET AL.: "Evaluation of a radioimmunoassay for alpha-1-acid glycoprotein to monitor therapy of cancer patients" JOURNAL NATIONAL CANCER INSTITUTE (JNCI), vol. 71, no. 1, 1983, pages 25-30, XP000909046 cited in the application abstract page 26, column 1, paragraph 2 ---	1-13
X	BIENVENU J. ET AL.: "Laser nephelometry of orosomucoid in serum of newborns: reference intervals and relation to bacterial infections" CLIN. CHEM., vol. 27, no. 5, 1981, pages 721-726, XP002139731 cited in the application page 721, column 2, paragraph 5 -page 722, column 1, paragraph 2 ---	1-13
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted]S 99/31284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KREMMER T. ET AL.: "Determination and analysis of human serum alpha-1-acid glycoprotein by liquid chromatographic methods" J. LIQUID CHROMATOGRAPHY, vol. 18, no. 6, 1995, pages 1207-1218, XP000909051 page 1209, paragraph 1 -page 1210, paragraph 2	1-13

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS

Cross-Reference to Related Application

This application claims the benefit of U.S. Provisional Application No. 60/114,520, filed December 30, 1998.

Field of the Invention

This invention relates to methods which are useful in the treatment of cancer with a pharmaceutical that is a member of the family of anti-neoplastic agents known as taxoids.

The family of anti-neoplastic agents known as taxoids are based on natural and modified compounds that have the taxone skeleton isolated from the yew tree (Taxaceae). Two particularly effective taxoids are paclitaxel, which is a natural product isolated from the Pacific yew (Taxus brevifolia), and docetaxel, which is a semisynthetic product derived from the needles of the European yew (Taxus baccata). The activities of these agents have been demonstrated in a wide variety of cancers, including breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. Other taxoids are being developed for cancer treatment also.

Examples

The following examples are representative of the practice of the invention.

Example 1

This example is illustrative of the present invention. It provides information stemming from a study of cancer patients who were treated with a taxoid (docetaxel) and concerning the relationship between AAG levels and a variety of physiological effects, including, for example, side effects. This study in its entirety is reported in applicant's U.S. provisional patent Application No. 60/114,520, filed December 30, 1998, which is incorporated herein by reference. The results of this study were published in Bruno et al., *Journal of Clinical Oncology*, Vol. 16, No. 1, p. 187-196 (1998).

The Patient Pool

Data were prospectively collected from patients entered in twenty-four Phase II open, non-randomized studies conducted from May 1992 to March 1994 to assess docetaxel clinical efficacy in a variety of tumor types including breast cancer, non small cell lung cancer, ovarian cancer, head and neck cancer, melanoma, renal cancer, colorectal cancer, gastric cancer, small cell lung cancer, and soft-tissue sarcoma. The studies were conducted in over 50 centers in Europe and three centers in the United States.

Criteria for eligibility included histology, at least one bidimensionally measurable lesion, adequate bone marrow reserve (absolute neutrophil count > 2,000/μL), adequate renal function (normal creatinine level) and liver function (total bilirubin level < 1.25 x ULN, SGOT (ALT) $\leq$2 x ULN or $\leq$3 x ULN in case of proven liver metastases). According to the tumor type, patients could have received various extents of prior treatment. The starting dose of docetaxel was either 75 mg/m^2 or 100 mg/m^2 given as a 1-hr infusion every 3 weeks. Dose reduction (25 %) or delay of subsequent courses were permitted, based on the degree of toxicity observed.

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(57) Abstract

A method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha–1–acid glycoprotein comprising observing the patient's level of alpha–1–acid glycoprotein, evaluating said level to determine the dosage of the taxoid to administer to the patient by comparing said level to a predetermined alpha–1–acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said evaluation, recommending the dosage of the taxoid to administer to the patient. Also, a method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid comprising observing the patient's alpha–1–acid glycoprotein level, comparing said level to a predetermined alpha–1–acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison, assessing the effect of continued treatment of the patient with respect to the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient. Also, a method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid comprising observing the patient's alpha–1–acid glycoprotein (AAG) level, comparing said level to a predetermined alpha–1–acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison recommending the dosage of said taxoid to administer to said patient to reduce the incidence or severity of side effects that the patient may experience during treatment with said taxoid.

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PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS

Cross-Reference to Related Application

This application claims the benefit of U.S. Provisional Application No. 60/114,520, filed December 30, 1998.

Field of the Invention

This invention relates to methods which are useful in the treatment of cancer with a pharmaceutical that is a member of the family of anti-neoplastic agents known as taxoids.

The family of anti-neoplastic agents known as taxoids are based on natural and modified compounds that have the taxone skeleton isolated from the yew tree (Taxaceae). Two particularly effective taxoids are paclitaxel, which is a natural product isolated from the Pacific yew (Taxus brevifolia), and docetaxel, which is a semisynthetic product derived from the needles of the European yew (Taxus baccata). The activities of these agents have been demonstrated in a wide variety of cancers, including breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. Other taxoids are being developed for cancer treatment also.

The taxoids appear to show a common mechanism of action based on promoting the assembly and inhibiting the disassembly of microtubules. This causes disruption of the microtubular network that is required for mitotic and interphase cellular functions thereby disrupting cell proliferation.

For a patient being treated with a taxoid, it is desirable to be able to predict the efficacy of the treatment and/or a suitable dosage level to administer to the patient. Efficacy can be characterized by the response to taxoid treatment and the survival of the patient. Suitable dosage levels relate to reducing or avoiding undesirable side effects that might be experienced by the patient while maintaining efficacy.

Reported Developments

Descriptions of clinical studies relating to the efficacy and side effects resulting from the clinically available taxoids, paclitaxel and docetaxel (TAXOTERE®) are provided in The Physicians Desk Reference, 52nd edition (1998) p762-766 (paclitaxel) and 2385-2389(TAXOTERE®). An extensive review of the clinical and preclinical profiles of docetaxel are presented in Cortes, J.E., and Pazdur, R.J., Clin. Oncol. 13(10) 2643-2655,(1995). An extensive review of the chemotherapy trials treating advanced breast cancer using the taxoids is provided in Clemens, M. et al., Eur. J. Cancer 33(13) 2183-21939(1997). A review of the pharmacokinetic parameters of paclitaxel and docetaxel and side effects experienced with the use of these taxoids is provided in Verweij, J. et al Ann. Oncol 5(6) p495-503 (1994).

Summary of the Invention

In accordance with the present invention, there is provided a method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein (AAG). The method includes observing the patient's level of AAG,

evaluating the AAG level to determine the dosage of a taxoid to administer to the patient by comparing the AAG level to a predetermined AAG level from a population of patients that have the same type of cancer and who are being treated with the taxoid at a common dosage, and, based on this evaluation, recommending the dosage of the taxoid to administer to the patient.

The taxoids can be, for example, docetaxel or paclitaxel. Examples of the type of cancers that can be treated include breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. An example of a preferred of a preferred embodiment of the present invention involves the treatment of non-small cell lung cancer.

Another aspect of the present invention is the provision of a method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid. This method includes observing the patient`s AAG level, comparing the AAG level to a predetermined AAG level derived from a population of patients having the same cancer and being treated with the taxoid at a common dosage, and, based on this comparison, assessing the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient.

In preferred embodiments, the patient is treated with a dosage of about 55 mg/m^2 to about 200mg/m^2 of the taxoid. In especially preferred embodiments of the invention, the patient is treated with about 55 to about 125 mg/m^2 of docetaxel or about 135 to about 175 mg/m^2 of paclitaxel.

Still another aspect of the present invention is the provision of a method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid. This method includes observing the patient's AAG level, comparing the AAG level to a predetermined AAG level derived from a population of patients

having the same cancer and treated with the taxoid at a common dosage, and, based on this comparison, recommending the dosage of the taxoid to administer to the patient to reduce the incidence or severity of side effects that the patient may experience during treatment with the taxoid.

Examples of side effects include neutropenia, infection, diarrhea, infusion related hypersensitivity reactions, allopenia, neurotoxicity, mucositis, stomatitis, sever asthenia and myalgia. Neutropenias include febrile neutropenia.

Brief Description of the Drawings

Figure 1 is a pharmacokinetic profile of the taxoid docetaxel in a representative patient with normal liver function (□) and a patient with elevated hepatic enzymes (■). Lines denote model predictions after Bayesian estimation.

Figure 2 is a docetaxel pharmacokinetic profile in a subset of 254 patients.

Figure 3 is a model-predicted probability of febrile neutropenia as a function of CLf for a patient with median AAG. Reference vertical lines denote normal CL (CLf=1) and 50% reduced CL (CLf=2).

Figure 4 shows survival curves in NSCLC patients with low (≤1.11 g/L, --), intermediate (1.12 to 1.84 g/L,·····), and high (≥1.85,-·-·-) base line AAG (/censored observation).

Detailed Description of the Invention

In connection with the development of this invention, it has been found that, for a cancer patient being treated with a taxoid, the patient's level of alpha-1-acid glycoprotein can be used to predict response to treatment, survivability, and side effects.

For background purposes there is set forth hereafter information relating to the taxoids and alpha-1-acid

glycoprotein(AAG). Following this information, methods for measuring AAG levels are described and the relationships between AAG levels and response to treatment, survivability, and side effects are discussed. With regard to side effects, methods for reducing the possibility of side effects by measuring a patient's AAG level prior to or during taxoid treatment and adjusting the dosage of the taxoid are discussed.

Taxoids

The present invention relates to treatment methods utilizing taxoids. A variety of taxoids may be used in the practice of the present invention. "Taxoid" as used herein refers to anti-neoplastic agents based on natural and modified compounds that have the taxane skeleton isolated from the yew tree. Preferred taxoids used in the practice of the invention are paclitaxel and docetaxel. Paclitaxel promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. Docetaxel binds free tubulin and promotes assembly of microtubules while simultaneously inhibiting the disassembly of the microtubules. This results in the stabilization of microtubules and inhibition of mitosis. The use of docetaxel is particularly preferred in the practice of the invention.

Paclitaxel has the chemical formula $C_{47}H_{51}NO_{14}$ and has a molecular weight of 853.9. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine (*Physician's Desk Reference, supra*)).

Docetaxel has the chemical formula $C_{43}H_{53}NO_{14}\bullet 3H_2O$ and has a molecular weight of 861.9. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-phenylisoserine,N-*tert*-butyl ester,13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate (see *Physician's Desk Reference, supra*).

In the practice of the present invention, any cancer that responds to treatment with the taxoids may be treated using the methods of the present invention. The taxoids are known to have activity against a variety of cancers, including, for example, breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. The taxoids have demonstrated activity against other types of cancers when used alone or in conjunction with other anti-neoplastic agents. The present invention is particularly well suited to patients undergoing treatment for non-small cell lung cancer (NSCLC).

Alpha-1-Acid Glycoprotein

There follows hereafter a description of the biochemical and genetic characteristics of alpha-1-acid glycoprotein (AAG) and a description of its function in the body. This is followed by a description of methods for measuring AAG levels and a discussion of the significance of AAG levels found in patients treated with a taxoid.

AAG Characteristics and Function

Alpha-1-acid glycoprotein (AAG), also called orosomucoid, is found in the seromucoid fraction of human blood plasma. Northern blot analysis of RNA extracted from a variety of tissues demonstrates preferential expression of AAG in the liver. The complete amino acid sequence of AAG is known (Schmid, K. et al., *Biochemistry*, 12, 2711-2724 (1973)) and the carbohydrate moiety has been also identified (Schmid, K. et al., *Prog. Clin. Biol. Res.*, 300, 7-2 (1989)). AAG consists of a single polypeptide chain of 183 amino acids with 21 substitutions possible having a molecular weight of approximately 21 kDa. Within the protein backbone there are five N-glycosylation sites for the attachment of oligosaccharides. There are believed to be at least seven alleles coding for AAG (Yuasa, I. et al., *Hum. Genet.*, 77, 255-258 (1987); Umetsu, K. et al., *Electrophoresis*, 9, 224-226 (1988)). Three phenotypes have been identified with autosomal co-dominant transmission. The variant forms result from amino acid substitutions. The structure and expression

of the genes coding for AAG have been described and the AAG locus has been mapped to the distal portion of the long arm of chromosome 9 (Dente, L. et al., Embo J., 6, 2289-2296 (1987); Eiberg, H. et al, *Clin. Genet.*, 23, 150-154 (1983)).

The normal function of AAG in the body is not completely understood. Based on *in vitro* studies, AAG may be involved in coagulation, phagocytosis, graft rejection, and wound healing. A review of the biological activities of AAG may be found in Kremer et al.(*Pharm. Rev.*, 40(1), 1-47 (1988)).

AAG is classified as an "acute phase protein" because the concentration of AAG increases following inflammatory stimuli. AAG synthesis also increases several fold during an acute phase response (Ricca et al., *J. Biol. Chem.*, 256, 11199-11202 (1981); Koj et al., *Biochem J.*, 206, 545-553 (1982); Koj et al., *Biochem J.*, 224, 505-514 (1984)). The major inducers of AAG synthesis are the cytokines interleukin-1 (IL-1) and interleukin-6 (IL-6), which act additively to induce transcription of the AAG gene.

Increased levels of AAG due to the acute response have been identified in cancer patients and it has been found that elevated levels of AAG can effect the efficacy of a variety of drugs.

AAG has long been known to bind drugs in plasma. AAG demonstrates high binding affinity for basic drugs (with pK values of 8 or higher). In addition, acidic and neutral drugs have also been shown to bind AAG. For an extensive review of the drug binding activity of AAG, see Kremer et al., (*Pharm. Rev.*, 40(1), 1-47 (1988)).

It is known also that the taxoids are bound by AAG. *In vitro* studies have demonstrated that up to 98% of the taxoid docetaxel is bound by plasma proteins, including AAG (see *Physician's Desk Reference, supra*). Similarly, *in vitro* studies of the binding of paclitaxel to serum proteins at paclitaxel concentrations from 0.1 to 50 μg/ml indicate that

89 to 98% of the paclitaxel is bound. Accordingly, it is expected that AAG has the ability to bind taxoids *in vivo*. Due to this binding interaction, the level of AAG in a patient's plasma becomes significant because, as more AAG is available, a greater percentage of an administered taxoid may be bound.

The present invention involves the relationship between AAG levels in a patient who is treated with a taxoid and response to treatment, survival, and side effects. Accordingly, the present invention involves the measurement of a patient's AAG level.

Methods for Measuring AAG Levels

A variety of bodily fluids and tissues may be used to evaluate a patient's AAG level in connection with the practice of the present invention. Methods of obtaining samples of bodily fluids and tissues are well known in the art. Blood plasma is the preferred bodily fluid used to determine AAG levels in the practice of the present invention. Guidance on obtaining blood samples in order to determine AAG levels may be found in Bienvenu et al., *Clinical Chemistry*, Vol. 27, No. 5, 1981, and in Ganz et al., *JNCI*, Vol. 71, No. 1, July 1983. In preferred embodiments utilizing blood plasma to determine AAG levels, a suitable volume of blood, for example, about 0.5 to about 0.2 ml is taken from a patient, preferably by venipuncture, and is centrifuged under sufficient conditions to isolate the plasma fraction for analysis of the AAG level.

A variety of methods known in the art can be used to determine a patient's AAG level. These methods include methods known for isolation and quantification of a protein. Suitable techniques for isolating and quantifying a protein may be found in a variety of sources, including e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, volumes I and II

(D.N. Glover ed. 1985); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994). Methods used to quantify AAG preferably utilize antibodies. A general overview of immunoassays is provided in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Pubs., NY (1988). Monoclonal or polyclonal antibodies specific for AAG can be used in immunoassays to quantify AAG. Monoclonal antibodies against AAG may be obtained from commercial sources, such as ICN Pharmaceuticals Inc. (Catalog No. 692011) or prepared using techniques known in the art. To produce monoclonal antibodies that bind AAG, hybridomas producing anti-AAG antibodies can be prepared and selected for as described in the literature. For example, mice (i.e., balb/c mice) can be immunized with AAG by intraperitoneal injection. After sufficient time has passed to allow for an immune response, the mice can be sacrificed and the spleen cells obtained and fused with myeloma cells using techniques well known in the art. The resulting hybridomas are then grown in a selective medium, and the surviving cells grown in such medium using limiting dilution conditions. After cloning and recloning, hybridomas can be isolated that secrete antibodies (for example, of the IgG or IgM class) directed against AAG. Immunoassays which can be used to quantitate AAG may include ELISA, competitive immunoassays, radioimmunoassays, indirect immunofluorescent assays and the like. Preferred methods for quantification of AAG include, but are not limited to rocket immunoabsorbant assays (Dewey et al., *J. Immunol.*, 144, 4392-8 (1990); radioimmunoassays (Ganz et al., *JNCI*, 71(1), 25 (July 1983); laser nephelometry (Bienvenu et al., *Clinical Chemistry*, 27(5), 721-726 (1981); and immunoassay in a Cobas Bio centrifugal analyzer (Verme et al., *Clinical Chemistry*, 34(1), 2316-2320 (1988)).

A highly preferred method for determining a patient's AAG level utilizes laser nephelometry as described in Bienvenu et al., *Clinical Chemistry*, 27(5), 721-726 (1981). In this method, a blood sample (0.4 ml to 0.2 ml) is collected by venous puncture, and the serum is removed after

centrifugation. A Behring Laser Nephelometer module I (Behringwerke, D-3550 Marburg/Lahn, Germany) is utilized for taking measurements. Samples, standards, and antisera are diluted with sterile isotonic saline solution and 100 μL of 101-fold diluted sample is mixed in a microcuvette with 200 μL of a fivefold diluted anti-orosomucoid antiserum (LN serum anti-orosomucoid (AAG) SAW; Behringwerke (or other commercially available or prepared antibody)). The cuvettes are shaken briefly and allowed to stand for 1 hour at room temperature, and the light scattered by the resulting antigen-antibody complexes is measured (in volts) with the nephelometer. A calibration curve is prepared by use of an 800mg/L standard solution of AAG, diluted to give concentrations of 40, 20, 10, 5, 2.5, and 1.25 mg/L and the concentration of the AAG in the sample is calculated based on its light scattering relative to the known standards.

The present invention includes within its scope situations in which a patient's AAG level is determined by a third party not involved in the predictive aspects of the present invention.

Significance of AAG Levels

The present invention is based in part on the discovery that, for cancer patients being treated with a taxoid, the patient's AAG level is a prognostic factor which allows predictions to be made regarding response to treatment, survival, and side effects. An explanation follows respecting the significance of AAG levels which are higher or lower than the norm or that fluctuate during treatment.

Typically blood or other body fluid samples are taken after a cancer has been diagnosed and during taxoid treatment. Samples may be taken at any point prior to or during the course of treatment. Blood samples obtained prior to manifestation of cancer may be useful in determination of a baseline AAG level in the absence of disease.

The range of AAG in normal individuals is from about 0.36 g/L to about 1.46 g/L. The level of AAG is often elevated in pathological states such as liver cirrhosis, renal disease and cancer. Individuals with cancer may have elevated AAG levels. For example, a study of patients (n=180) having NSCLC had levels of AAG ranging from about 0.84 to about 2.71 g/L, with a median of 1.42 g/L. Approximately half of the patients had an AAG level exceeding the maximum AAG level (1.46 g/L) seen in healthy subjects.

Methods known in the art can be used to evaluate and classify ranges of AAG concentrations in "taxoid-treated" patients with various types of cancer. A sample population of patients having a particular type of cancer who are being treated with a particular taxoid at a common dosage may be studied to quantify the relationship between AAG levels and response to treatment, survival and side effects. The term common dosage refers to a population all of which are receiving the same dosage of a taxoid, for example, a dosage of about 100 mg/m^2. Given a common dosage, patients within a population may receive differing absolute amounts of a taxoid depending on their size. The dosage of a taxoid being administered to a population will depend on the type of cancer and the taxoid being used. Generally speaking, it is believed that the taxoid dosage will fall within the range of about 55 to about 200 mg/m^2, but may be higher or lower, as conditions warrant. A typical dosage range will usually be about 75 mg/m^2 to about 175 mg/m^2. For a given type of cancer, the range of AAG concentrations in the population may be obtained and defined as high, intermediate, and low, using data from the population of patients and standard statistical methods. The AAG concentration ranges in the population constitute "predetermined" AAG levels which are then used for comparison and evaluation of an individual patient's AAG level. For example, for a population of patients, the 25% quantile of the AAG distribution in the population can be classified as the low level and the 75% quantile can be classified as the high level, with the >25% quantile to <75% quantile being classified as intermediate level.

Accordingly, in the practice of the present invention, the AAG concentration is determined using any of the assays described above, preferably the Bienvenu et al. Laser Nephelometry assay. Once the AAG level is known, the patient may be classified as having a high, intermediate, or low AAG level according to the quantile level into which the patient's AAG level falls. After the patient's AAG level has been classified, this level may be compared with observations on the relationship between AAG level and response to treatment, survival, and side effects for a patient population having the same type of cancer and being treated with the same type of taxoid.

Response to Treatment

The term "response to treatment" refers to whether a patient responds to treatment according to the standard criteria for partial response (PR=50% reduction in tumor) and complete response (CR= a complete reduction in tumor) as defined by the National Cancer Institute. Non-response is defined as patients with minor responses (< 50 % reduction in tumor size) evaluable disease, stable disease and patients with disease progression.

The present invention provides methods for assessing the effect of treatment of a given cancer with a given taxoid based on observing the patient's AAG level, comparing this level to a predetermined AAG level derived from a population of patients having the same type of cancer and being treated with the same taxoid at a common dosage, and assessing the effect of continued treatment.

The chances of a patient responding to treatment with a taxoid relates to the patient's AAG level. It has been found that there is a significant increase in the chance of a patient responding to taxoid treatment for a patient who exhibits a low AAG level. In general, if a patient has a low AAG level there is an increased chance of response to taxoid treatment relative to the chance of response for a patient with high AAG levels.

Accordingly, a blood sample can be obtained from a patient and the observed AAG level classified as high or low according to the guidance provided hereinabove. The AAG level is evaluated in order to consider recommending an adjustment in the taxoid dosage and/or supplementing treatment with additional chemotherapeutic, surgical or radiation treatments to increase the chance of response to treatment. Based on the AAG level, the patient can be classified as having an increased chance of response if the patient has a low AAG concentration. For a patient with a high AAG level, the patient's response may be considered to be reduced relative to patients having low AAG concentrations.

If a quantitative characterization of the relationship between AAG and response rate is desired for a particular type of cancer, one of skill in the art can readily obtain blood samples from a population of patients having a given type of cancer and follow the guidance provided in the Examples below to further define the correlation between AAG levels and response to treatment with a taxoid.

Survival

The term "survival" is defined as the length of the patient's life from the time of the first infusion of a taxoid dosage to the date of death. The present invention provides methods of assessing the effect of treatment as it relates to survival for a patient who has cancer and who is being treated with a taxoid. The method involves observing an individual patient's AAG level, classifying the AAG level as low, intermediate or high AAG compared to predetermined AAG levels in a patient population having the same type of cancer under treatment with the same taxoid at a common dosage and assessing the effect of continued treatment in order to predict the patient's survival. The AAG level is evaluated in view of a population of patients having the same type of cancer to consider recommending an adjustment in the taxoid dosage and/or supplementing the treatment with additional chemotherapeutic, surgical or radiation treatment

to prolong survival. In accordance with the present invention, it has been found that patients having low AAG levels will be expected to survive longer than patients with high AAG levels.

If a quantitative description of the relationship between AAG level and survival is desired for a particular type of cancer, one of skill in the art may obtain blood samples from a population of patients having that type of cancer and being treated with the same taxoid at a common dosage and may follow the protocol presented in the Examples below to further define the relationship between AAG level and survival.

In addition to survival, the methods of the present invention are also useful in predicting time to progression. Time to progression is calculated from the first administration of the taxoid to the date of progression as discussed in the examples below. Studies of patients with NSCLC demonstrated that patients with low AAG levels ($\leq$ 1.09 g/L) had a longer time to progression (18 weeks) versus 9.7 weeks for patients with high AAG levels ($\geq$ 1.92 g/L). Accordingly, the methods described above for survival may also be used with regard to time to progression.

Side Effects

The term "side effects" refers to adverse effects produced by a drug such as a taxoid, especially on a tissue or organ system other than the one sought to be treated with the drug. Use of the taxoids can result in a variety of side effects, including, for example, neutropenia, infusion-related hypersensitivity reactions, allopecia, neurotoxicity, mucositis, infections, stomatitis, diarrhea, severe asthenia, fluid retention and myalgias.

The nature and severity of the side effects due to the use of a given taxoid will depend on a variety of factors, including the specific taxoid used, the dosage, the overall

dosing regimen, the presence of other drugs, and factors relating to the patient's physiological state.

The side effects specific to paclitaxel and taxotere are well documented (see *Physician's Desk Reference, supra*, Cortes and Pazdur, *Journal of Clinical Oncology*, vol. 13, No. 10, 2643-2655 (October 1995)). The major dose-limiting side effect of paclitaxel is neutropenia. Other side effects include dose dependent mucositis and peripheral neuropathy, cardiac rhythm abnormalities, arthralgias/myalgias, hypersensitivity reactions, alopecia, nausea and vomiting. The major dose limiting side effect of docetaxel is neutropenia. Other side effects include paresthesias, hypersensitivity reactions, alopecia, skin reactions, fluid retention, nausea, vomiting and diarrhea. A discussion of the side effects experienced with paclitaxel and docetaxel (TAXOTERE®), may be found in the *Physician's Desk Reference, supra*. These side effects may be defined and graded using the common toxicity criteria of the U.S. National Cancer Institute or COSTART classification. The patient's AAG level may be used to predict the possibility of a variety of side effects, in particular, grade 4 neutropenia, infection and grade 3 diarrhea.

The present invention provides methods for assessing the effect of treatment as it relates to side effects for a patient who has cancer and who is being treated with a taxoid. The method involves observing the patient's AAG level, classifying the AAG level as high, intermediate or low compared to predetermined AAG levels derived from a population of patients having the same type of cancer and being treated with the same taxoid at a common dosage, and based on this comparison assessing the side effects that may be experienced by the patient.

The present invention also provides a method for assessing whether a patient who has cancer and who is to be treated with a taxoid will experience side effects. The method involves observing the patient's AAG level prior to

treatment and comparing this level to a predetermined AAG level derived from a population of patients having the same type of cancer and being treated with the same taxoid that is to be used in treating the patient, and, based on this comparison recommending a dosage of the taxoid that will reduce or eliminate side effects that may be experienced by the patient, while providing an improvement or cure in the patient's condition.

The odds of experiencing side effects resulting from taxoid treatment can be predicted based on AAG levels. The relationship between AAG levels and the occurrence of side effects is believed to relate to the AAG-taxoid binding interaction. In accordance with the present invention, it has been found that patients with high AAG levels are less likely to experience adverse side effects than patients with low AAG levels.

If a quantitative characterization of the relationship between AAG and side effects is desired for a particular type of cancer, one of skill in the art can readily obtain blood samples from a population of patients having a given type of cancer and follow the guidance provided in the Examples below to further define the relationship between AAG levels and side effect(s) due to treatment with a given taxoid.

Determination of Dosage Levels

Based on the ability to predict response to treatment, survival, and side effects, the present invention may be used to adjust the dosage of a taxoid being administered to a patient. Accordingly, the present invention provides methods for determining the dosage of a taxoid to administer to a patient being treated for a cancer. These methods involve observing the patient's level of AAG and evaluating the AAG level to determine the dosage of the taxoid to administer to the patient by comparing the patient's AAG level to a predetermined AAG level in a population of patients who have the same type of cancer and who are being treated with the same taxoid at a common dosage. Based on this information, a

recommendation can be made on the dosage of taxoid to give to the patient. With regard to response rate, a patient who has a high AAG level and who is predicted to have a decreased chance of responding to treatment may have their taxoid dosage increased. Similarly, a patient who has a high AAG level and who is predicted to have a reduced length of survival may have their taxoid dosage increased. Given that patients with high levels of AAG are also less likely to experience side effects, it may be possible to increase the AAG dosage for these patients without a corresponding increase in the possibility of side effects.

Given the correlation between AAG levels and side effects, one method for reducing the possibility of side effects is to determine the patient's AAG level and to adjust the taxoid dose so as to reduce the possibility of side effects.

Accordingly, the present invention also provides methods for reducing the chance of a cancer patient's experiencing side effects from a taxoid by observing the patient's level of AAG prior to treatment and classifying the patient's level of AAG as high or low. If the patient has a low AAG level, the dose of a taxoid to be administered to the patient may be reduced so as to reduce or eliminate any possible side effects.

The dosage levels for taxoids are specific to the particular taxoid being used and the cancer being treated. Dosage recommendations for the clinically available taxoids are provided with the products and may also be found in the Physicians' Desk Reference and in the scientific literature. Dosage recommendations for the taxoid docetaxel range from about 55 mg/m^2 to about 125 mg/m^2. These dosages are usually administered intravenously over 1 hour every three weeks. Dosage recommendations for the taxoid paclitaxel range from about 135 mg/m^2 to about 175 mg/m^2. These dosages are usually administered intravenously over 3 hours every three weeks.

Guidance for adjusting taxoid dosage based on actual side effects may be found in the *Physician's Desk Reference*, 52nd ed., (1998) (for TAXOL® (paclitaxel) see p762-766 and for TAXOTERE® (docetaxel) see p2385-2389). These recommended adjustments based on actual side effects may be used as a guide to adjusting dosage based on predicted side effects.

With regard to paclitaxel, patients who have low AAG levels and would be predicted to experience neutropenia or other side effects such as infection or grade 3 diarrhea during paclitaxel therapy may have their paclitaxel dosages reduced by about 5 to about 35%, preferably by about 10 to about 30%, even more preferably from about 15 to about 27%.

With regard to docetaxel (Taxotere®), patients who would be predicted to experience neutropenia, including febrile neutropenia or other side effects may have their docetaxel dosage reduced by about 5 to about 35%, preferably by about 10 to about 30%, even more preferably about 15 to about 27%. If the side effects actually occur, the dosage may be further decreased.

In general, the taxoid dosage may be adjusted upwardly, or downwardly, based on actual side effects and response to treatment.

Description of an Embodiment of the Invention

The methods of the present invention are illustrated in the Examples below which describe a study involving NSCLC cancer patients who were treated with the taxoid docetaxel (Taxotere®). The study involved a determination of the relationship between AAG levels and response to treatment, survival, and side effects.

The study included 180 NSCLC patients who were enrolled in six Phase II studies of 100 mg/m^2 of docetaxel.

The AAG levels of the patients were determined and classified into high (≥1.85 g/L (75 percentile and above)),

intermediate (1.12 to 1.84 g/L (26 percentile to 74 percentile), and low (≤1.11 g/L (25 percentile and below) levels.

The general relationship between AAG levels and response, survival, and side effects that were observed in NSCLC patients treated with the taxoid docetaxel can be summarized as follows: (a) patients with low AAG levels have a greater response rate to treatment with a taxoid than patients with high AAG levels; (b) patients with low AAG levels being treated with a taxoid survive longer than patients with high AAG levels; and (c) patients with low AAG levels will be more likely to experience adverse side effects from taxoid treatment relative to patients with high AAG levels.

As presented in the Examples below, the study shows that a patient having a low AAG level (≤1.11 g/L) had a response rate of 41.3% compared to a 15.9% response rate for patients with high (≥1.85 g/L) AAG levels. Accordingly, a patient's AAG level can be determined using the methods described above, preferably the Bienvenu et al. method, and the patient's observed AAG level may be compared to the population's predetermined AAG levels and the patient's AAG level classified into a low, intermediate, or high category. If the patient has a low AAG level, it can be predicted that the patient will have an increased chance of response to treatment. Similarly, if the patient's AAG level falls into the high AAG level category, it can be predicted that the patient will have a reduced chance of responding to treatment with a taxoid. Based on these predictions, treatment options may be considered, including, for example, maintaining the treatment at the current taxoid dosage, adjusting the taxoid dosage and/or expanding the treatment to include additional chemotherapeutic, surgical, or radiological treatment.

With regard to survival, patients having a low AAG level (≤1.11 g/L) had a median survival of 15.6 months. Patients having an intermediate AAG level (1.12 to 1.84 g/L) of AAG

had a median survival of 9.2 months and patients with a high level (≥1.85 g/L) of AAG had a median survival of 5.5 months. Accordingly, a patient's AAG level can be determined using the methods described above, preferably the Bienvenu et al. method and the patient's observed AAG level may be classified as having a low, intermediate, or high level of AAG compared to the population's predetermined AAG levels. Based on the patient's AAG level, the patient may be predicted to have a period of survival measured from the initiation of taxoid treatment to be of long, intermediate, or short duration. For example, a patient having a low AAG level would be expected to have a longer survival than a patient with intermediate or high levels or AAG. For patients with intermediate or high AAG levels, treatment options may be considered, including, for example, maintaining the treatment at the current taxoid dosage, adjusting the taxoid dosage and/or expanding the treatment to include additional chemotherapeutic, surgical, or radiological treatment.

With regard to side effects, as the AAG level varied from low (≤1.11 g/L) to high (≥1.85 g/L), there was approximately a 50% reduction in the odds of experiencing an adverse side effect (febrile neutropenia or infection or grade 3 diarrhea). Accordingly, a patient's AAG level can be determined using any of the methods described above, preferably the Bienvenu et al. method, and the patient's observed AAG level can then be classified as low, intermediate, or high AAG level compared to the population's predetermined AAG levels. If the patient's AAG level is in the low range, it can be predicted that the patient will have an elevated chance of experiencing side effects. If the patient is predicted to have an elevated chance of experiencing side effects, consideration can be given to lowering the patient's dose of the taxoid so as to reduce the chances of undesirable side effects. Reduction of the dosage of the taxoid must be balanced against reduction in the efficacy of treatment.

Examples

The following examples are representative of the practice of the invention.

Example 1

This example is illustrative of the present invention. It provides information stemming from a study of cancer patients who were treated with a taxoid (docetaxel) and concerning the relationship between AAG levels and a variety of physiological effects, including, for example, side effects. This study in its entirety is reported in applicant's U.S. provisional patent Application No. 60/114,520, filed December 30, 1998, which is incorporated herein by reference. The results of this study were published in Bruno et al., *Journal of Clinical Oncology*, Vol. 16, No. 1, p. 187-196 (1998).

The Patient Pool

Data were prospectively collected from patients entered in twenty-four Phase II open, non-randomized studies conducted from May 1992 to March 1994 to assess docetaxel clinical efficacy in a variety of tumor types including breast cancer, non small cell lung cancer, ovarian cancer, head and neck cancer, melanoma, renal cancer, colorectal cancer, gastric cancer, small cell lung cancer, and soft-tissue sarcoma. The studies were conducted in over 50 centers in Europe and three centers in the United States.

Criteria for eligibility included histology, at least one bidimensionally measurable lesion, adequate bone marrow reserve (absolute neutrophil count > 2,000/μL), adequate renal function (normal creatinine level) and liver function (total bilirubin level < 1.25 x ULN, SGOT (ALT) $\leq$2 x ULN or $\leq$3 x ULN in case of proven liver metastases). According to the tumor type, patients could have received various extents of prior treatment. The starting dose of docetaxel was either 75 mg/m^2 or 100 mg/m^2 given as a 1-hr infusion every 3 weeks. Dose reduction (25 %) or delay of subsequent courses were permitted, based on the degree of toxicity observed.

Most of the patients who registered (721/936, 77%) were sampled and among them, 81 were not considered evaluable for the study for the following reasons: not sampled at the first course (n=12, 1.7%); lack of documentation of samples (n=32, 4.4%); samples lost during transfer from the clinical sites to the analytical laboratory (n=18, 2.5%); or during assay procedure (n=19, 2.6%). Overall, 640 patients (89% of patients sampled, 68% of patients treated) were evaluable at first course.

Measurement of AAG Levels

AAG levels were determined by a variety of methods, primarily by the Bienvenu et al. laser nephelometry method. (See Bienvenu et al. Clinical Chemistry, 27(5), 721-726 (1981) and Example 3.)

Sampling Strategy

The aim of the sampling strategy in connection with taking blood samples from the patients was to define the full pharmacokinetic profile over the population, the so called "full screen" approach (Sheiner LB, Benet L Z: Premarketing observational studies of population pharmacokinetics of new drugs. Clin Pharmacol Ther 38: 481-487, 1985), by drawing a few samples per patient and varying (randomizing) the sampling times among patients (Hashimoto Y, Sheiner LB: Designs for population pharmacodynamics : Value of pharmacokinetic data and population analysis. J Pharmacokinet Biopharm 19: 333-353, 1991).

Recognizing the goal of individual estimates, the sampling strategy design was based on optimal individual sampling times computed using preliminary population PK parameter estimates obtained from Phase I data (Launay-Iliadis MC, Bruno R, Cosson V, et al: Population pharmacokinetics of docetaxel during Phase I studies using nonlinear mixed-effect modeling and nonparametric maximum-likelihood estimation. Cancer Chemother Pharmacol 37: 47-54, 1995). The sampling times were D-optimal (D'Argenio DZ: Optimal sampling times for pharmacokinetic experiments. J

Pharmacokinet Biopharm 9:739-756, 1981) and were computed using the APIS package, version 3.03a (Iliadis A, Brown AC, Huggins ML: APIS : A software for model identification, simulation and dosage regimen calculations in clinical pharmacokinetics. Comput Methods Programs Biomed 38: 227-239, 1992). Recognizing the goal of population estimates, separate sampling schedules, each consisting of early, mid and late time samples, were used to assure that the population PK samples were well spread across the available sampling time range.

There were 6 D-optimal sampling times (OST) for a three-compartment PK model (involving 6 parameters). OSTs were computed over a 0-24 hours observation interval. The estimated times (h:min) were: 0:30 (mid-infusion) or 1:00 (end of infusion), 1:15, 1:45, 3:45, 8:20 and 24:00.

The blood-sampling strategy consisted of four different sampling schedules (Table 1 below) which were assigned randomly to patients at study entry. Each schedule consists of 3 sampling times ranging between mid-infusion and 6 hours (5 hours post infusion). The first sample was always taken during the infusion, either mid infusion or just (5 minutes) before the end of the 1 hour-infusion. The two other samples were drawn within 5 hours after the end of infusion. Six hours was the maximum observation time in order to comply with outpatient status. However, when possible (e.g. for inpatients), one point could be replaced by a late sample drawn any time between 12 and 24 hours. A predrug sample (optional) was also requested to check the absence of analytical interference in patient plasma.

TABLE 1

SAMPLING STRATEGY IMPLEMENTED IN PHASE II STUDIES

Sampling Schedule No.	Sampling Times		
	1	2*	3
	During Infusion	After Infusion	
		Minutes	Hours
1	5 minutes before end	10	2
2	30 minutes after start	20	3
3	5 minutes before end	30	4
4	30 minutes after start	60	5

* When possible, this sample will be replaced by a blood sample obtained at a later time, i.e., any time between 12 and 24 hours post infusion.

A pharmacokinetic case report form (PK CRF) was designed to document actual sampling times as well as actual time of beginning and end of infusion. In some patients experiencing infusion-related hypersensitivity reactions, administration was interrupted and then resumed shortly after (e.g. 30 minutes). Actual times of starting and stopping the 2nd infusion were also documented on the PK CRF. Docetaxel was assayed in plasma samples using high performance liquid chromatography and UV detection after solid-phase extraction (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992) in 2 different cross-validated centers.

Pharmacokinetic Data Analysis

The collected data permitted elaboration and validation of a population pharmacokinetic model relating docetaxel clearance to patho-physiologic factors. This analysis has recently been reported (Bruno R., Vivier N., Vergniol J.C., et al: A population pharmacokinetic model for docetaxel (Taxotere®) : Model building and validation. J Pharmacokinet Biopharm 24:153-172, 1996). Population parameters from this analysis were used as prior information to estimate each

individual's pharmacokinetic parameters from his plasma concentrations using Bayesian estimation as implemented in the NONMEM computer program (version IV, level 2.0) (Beal S L, Boeckman AJ, Sheiner LB. NONMEM. User's Guide Part I to VI. University of California at San Francisco, San Francisco, 1988 - 1992).

The PK model was a three-compartment structural model with first-order elimination. The basic parameters were elimination clearance (CL, L/h), volume of distribution of the central compartment and intercompartmental rate constants. The inter-patient variability of PK parameters was modeled as (e.g. for CL):

$$CL_j = \hat{CL}_j \exp(\eta_{jCL})$$

where η_{jCL} denotes the (proportional) difference between the true parameter (CL_j) of individual j and the typical value in the population $\hat{CL}_j$ according to covariable values affecting $\hat{CL}$ for the j^{th} individual. Residual variability was modeled as proportional, consistent with the constant coefficient of variation of the assay measurement error (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992).

Individual plasma clearance (CL_j), area under the plasma concentration-time curve (AUC_j), peak plasma level, and time that plasma levels were greater than given threshold levels were used as measures of drug exposure.
CL_j was directly estimated by the Bayesian CL after fitting. Based on the estimate of CL_j, the following clearance factor (CLf) was generated:

$$CLf_j = (\text{mean CL}) \; / \; CL_j$$

Note that CLf_j is inversely proportional to CL_j : it takes values less than 1 for patients with clearance greater than the mean, and values greater than 1 for patients with clearance less than the mean (e.g. 2.0 for a 50 % decrease in clearance). Use of this derived parameter facilitates the

interpretation of pharmacokinetic/pharmacodynamic (PK/PD) models in term of clearance changes, as discussed below. AUC_j is computed as :

AUC_j ($\mu g \cdot h/mL$) = $Dose_j$ (mg) / CL_j (L/h)

Peak plasma level was taken to be the model predicted concentration at the end of infusion. Duration of exposure to plasma levels greater than 0.16 μg/mL (0.20 μM) ($t_{0.20}$), 0.080 μg/mL (0.10 μM) ($t_{0.10}$) and 0.040 μg/mL (0.05 μM) ($t_{0.05}$) was computed from estimated parameters using the implicit equation solver of EXCEL spread sheet, version 5 (Microsoft Corporation).

PK/PD analysis was conducted using as independent variables individual estimates, CLf_j, other exposure parameters (see above) and several other covariables related to the patient's patho-physiological status (demographics, disease spread) and extent of prior treatment. Docetaxel dose (mg/m^2), either given at first course or cumulative, was also considered as an independent variable measuring drug exposure.

Objective response rate, time to first response, and time to progression were selected as the efficacy endpoints (dependent variables). Only data from patients with breast cancer and non-small cell lung cancer (NSCLC) were analyzed. Assessment of tumor response was made every six weeks according to WHO criteria. Objective responses (complete responses (CR) and partial responses(PR)) were confirmed after a minimum of 4 weeks and were reviewed by an independent panel. Time to first response was calculated from the first docetaxel infusion up to the date of the first objective response either CR or PR whichever occurred earlier. Time to progression was calculated from the first docetaxel infusion up to the date of progression.

For safety, the following endpoints were considered among all tumor types:

- Neutropenia (NCI Grade) at first course.
- Febrile neutropenia at first course. Febrile neutropenia was defined as fever > 38°C (NCI grade ≥ II) with a

concomitant NCI grade 4 neutropenia (neutrophil count < 500/ μL) requiring antibiotics and/or hospitalization.
- Time to onset of fluid retention calculated from the first docetaxel infusion up to the date of the first sign and/or symptom of fluid retention (peripheral edema, pleural or pericardial effusions, ascites or weight gain).

Logistic regression was used to relate categorical endpoints, such as response rate and neutropenia grade, to the independent variables, while Cox regression was used for time to first response, time to progression and time to onset of fluid retention. Dose was the only time-dependent covariate in the Cox model. Model development involved stepwise inclusion and deletion of covariates. Significance levels for variable entry or removal at each step was $P < 0.10$; however, a final elimination pass, using $P < 0.05$ was used to determine the covariates kept in the final model. The median time to onset of fluid retention was estimated using the Kaplan-Meier method. Analyses were carried out using the SAS software (SAS version 6.11; SAS Institute Inc., Cary, NC).

Discussion of Results

Typical individual PK profiles are shown in Fig. 1 illustrating two of the four sampling schedules. The full population PK profile achieved by varying the sampling scheme across patients is illustrated in Figure 2 (data from a subset of 254 patients). This profile comprises 716 data points, that is, a mean of 2.8 per patient (range 1 to 5). Overall, a fair number of late samples was obtained (67 samples over 50 patients).

Patient characteristics at baseline

Patient characteristics are summarized in Table 2. Median age was 56 years, 42% were males and 58% females, 231 patients (36%) had breast cancer and 189 (30%) had NSCLC. Thirty-two percent of the patients were asymptomatic (WHO performance status of 0), whereas performance status of 1 and 2 were reported in 54% and 14% of the patients respectively. Thirty-three percent of patients had ≥ 3 organs involved, 82

% had visceral metastases, 35 % had liver metastases and 45% had previously been treated with chemotherapy. Most of the patients (95%) received 100 mg/m² as initial dose. Initially no premedication was used. Various premedication regimens (anti-H1 ± anti-H2 and/or corticosteroids either short term (≤ 2 days) or long term (≥ 3 days)) were subsequently given in some studies to prevent hypersensitivity reactions and fluid retention occurring during treatment. Few patients (n=25, 3.9%) received the five-day dexamethasone, presently recommended, premedication (8 mg orally twice daily starting the day before docetaxel administration).

TABLE 2

PATIENT CHARACTERISTICS AND DOCETAXEL EXPOSURE (N=640)

	COUNT		MEDIAN	5% TO 95% PERCENTILE
	NO.	%		
Age, years			56	38-71
Sex Male Female	 270 370	 42 58		
WHO performance status 0 1 2	 202 342 90	 32 54 14		
Total protein (g/L)			71	59-81
Albumin (g/L)			41	31-48
AAG (g/L)			1.34	0.76-2.59
Elevated liver enzymes	26	4.1		
Tumor type Breast NSCLC Other	 231 189 220	 36 30 34		
Disease spread No. of disease sites ≥3 Visceral involvement (yes) Liver metastasis (yes)	 214 522 221	 33 82 35		
Prior treatments Chemotherapy (yes) No. of prior regimens (≥2)	 289 110	 45 17		
Taxotere treatment/exposure Initial dose (mg/m²) 75 100	 31 609	 5 95		
CL (L/h)			36.3	17.5-59.3
CLf			1.02	0.622-2.11

	COUNT		MEDIAN	5% TO 95% PERCENTILE
	NO.	%		
AUC (μg-h/mL)			4.81	2.93-9.52
Peak (μg/mL)			3.26	1.93-5.76
$t_{0.20}$ (hours)			2.41	1.52-6.16 (0.858†)
$t_{0.10}$ (hours)			3.65	2.24-16.7 (0.856†)
$t_{0.05}$ (hours)			9.60	3.38-30.7 (0.838†)
Premedication				
None	252	39		
Recommended (5 days dexamethasone)	25	5		
Other	363	57		

* Patients with concomitant elevations of transaminases (>1.5 x ULN) and alkaline phosphatase (>2.5 x ULN).

† Correlation coefficient with AUC.

Individual PK parameter estimates

Individual estimates of PK and exposure parameters are given in Table 2. The continuous lines in Fig. 1 denote fits of patient data obtained using Bayesian estimation. In this large patient population, median clearance was 36.3 L/h which is a value very close to the value of 35.6 L/h previously estimated from Phase I data (Launay-Iliadis et al. Cancer Chemother Pharmacol 37:47-54 (1995)) and varied from 17.5 L/h to 59.3 L/h (5% to 95% percentile range). Representative exposure parameters were AUC: 4.81 μg·mL/h and peak: 3.26 μg/mL. Duration of exposure greater than threshold levels varied from 2.41 hours (0.20 μmol/L) to 9.60 hours (0.05 μmol/L). All of the measures of duration of exposure were strongly correlated with AUC $r \geq 0.838$, Table 2).

Pharmacokinetics/Pharmacodynamics - Efficacy

No significant relationship was found between any estimate of docetaxel exposure and either objective response rate, time to first response or time to progression in breast cancer (201 evaluable patients, response rate: 56%). The number of disease sites was a significant predictor of response for all endpoints ($p<0.05$), baseline alpha-1-acid glycoprotein level (AAG) and number of prior chemotherapy

regimens were additional predictors ($p<0.005$) of time to progression.

Regarding NSCLC (151 evaluable patients, response rate : 29%), docetaxel AUC at first cycle was a significant predictor ($p=0.0232$) of time to progression after adjusting for other covariates (see Table 3). AUC was the only measure of docetaxel exposure to reach statistical significance. The median time to progression was 99 days (95% confidence interval: 84 - 121 days). According to this model, the risk of progression is decreased by 11 % per unit AUC and by 43 % for 5 AUC units (e.g. from the median to about the 95 percentile in this population). In addition, duration of exposure over 0.10 μmol/L was the only measure of exposure to reach borderline statistical significance ($p\sim0.10$) in predicting either response rate or time to first response. Of note, baseline AAG was a significant predictor of response for all endpoints ($P<0.005$).

TABLE 3

NSCLC: COX REGRESSION MODEL FOR TTP (N=151)

PREDICTOR	P	RISK RATIO	95% CI
Cumulative dose*	.0002	0.997	0.995-0.998
No. of disease sites	.0011	1.293	1.109-1.507
AAG	.0022	1.757	1.225-2.518
Performance status	.0177	1.483	1.071-2.055
AUC	.0232	0.891	0.807-0.984

Note: Progression occurred in 84% of patients (127 of 151).
Abbreviation: CI, confidence interval.

* Time-dependent covariate.

Neutropenia

Neutropenia was analyzed at first course in 582 patients. Most of the patients (375/582, 64%) experienced grade 4 neutropenia. Several strong predictors of grade 4

neutropenia were identified including the various measures of docetaxel exposure with Clf, AUC and $t_{0.20}$ having the strongest effects (P<0.0001). After adjustment for the other covariates in the model, dose no longer had a significant effect. Clearance factor, CLf was retained in the final model (Table 4 below) since it greatly facilitates the interpretation of the model in terms of clearance change. The incidence of neutropenia grade 4 was related to the baseline neutrophil count (P=0.0002) and the number of previous regimens (P=0.0002) as expected. Baseline AAG level and first course exposure were the most significant predictors (P<0.0001). The higher the AAG level at baseline, the lower the odds of experiencing grade 4 neutropenia during the first course of treatment. According to the logistic regression model, a 1-g/l increase of baseline AAG (for example, from the median to about the 95 percentile in this population) results in a 83% decrease in the odds of experiencing grade 4 neutropenia. The effect of drug exposure change is the opposite with a 430% (4.3 fold) increase of the odds of grade 4 for a 1 unit increase in CLf. A 1 unit increase in CLf corresponds to a 50% decrease of clearance which is also a change from the median to the 95th percentile in this population).

TABLE 4

LOGISTIC REGRESSION MODEL FOR GRADE 4 NEUTROPENIA (N=582)

PREDICTOR	P	ODDS RATIO	95% CI
AAG	<.0001	0.17	0.10-0.29
Clf	<.0001	4.26	2.46-7.39
Baseline count	.0002	0.84	0.77-0.92
No. of previous regimens	.0002	1.72	1.30-2.29

Note: Incidence, 64% (375 of 582 patients)

Febrile neutropenia was observed in 26 of the 582 patients (4.5%) at first cycle. The model for this endpoint

was similar to that for neutropenia grade 4 with exposure (CLf) and AAG being the only significant predictors (Table 5 below). In this model, change of exposure due to a 50% decrement in clearance would result in a 300% (3.0 fold) increase in the odds of febrile neutropenia. The model-predicted probability of febrile neutropenia as a function of Clf (AAG fixed at the median) is illustrated in Figure 3.

TABLE 5

LOGISTIC REGRESSION MODEL FOR FEBRILE NEUTROPENIA (N=582)

PREDICTOR	P	ODDS RATIO	95% CI
Clf	.0012	3.03	1.55-5.93
AAG	.0056	0.28	0.12-0.69

Note: Incidence, 4.7% (26 of 582 patients).

Fluid retention

Fluid retention occurred in 53% of 631 evaluable patients. The median time to onset was 85 days (95% confidence interval: 81 to 92 days). Patients with breast and ovary carcinoma had disease related baseline symptoms resulting in a higher baseline risk than patients with other tumor types. The analysis was stratified, therefore, by tumor type with breast and ovary combined and other tumor types combined. Fluid retention incidence was 73% (172 of 236) in patients with breast or ovary tumors and 41% (163 of 395) in patients with other tumor types. Of note, few patients (n=25, 4%) received the presently recommended 5-day dexamethasone premedication in this population since this premedication was only recommended after the majority of these patients had been treated.

Owing to the cumulative nature of docetaxel induced fluid retention, dose was treated as a time-dependent covariate in the analysis. Cumulative dose was the most important predictor in the final Cox regression model (Table 6 below). However, several other baseline covariates had independent predictive power including AAG and total protein

levels. Drug exposure at first course was also highly significant in predicting the time to onset of fluid retention, after adjustment for the effect of cumulative dose. The duration parameter, $t_{0.20}$ was the most significant (P=0.0029) measure of exposure for this regression.

TABLE 6

COX REGRESSION MODEL FOR TIME TO ONSET OF FLUID RETENTION

(N=631)

PREDICTOR	P	RISK RATIO	95% CI
Cumulative dose*	<.0001	1.005	1.003-1.007
$t_{0.20}$	.0029	1.087	1.029-1.148
Total protein	.021	0.980	0.964-0.997
AAG	.014	0.746	0.591-0.942

Note: Incidence, 53% (335 of 631 patients).

Stratification: breast/ovary-236 patients/incidence, 73%; other-395 patients/incidence, 41%.

*Time-dependent covariate.

According to the model, the risk of experiencing fluid retention at any time is increased by 64% for each additional cycle at 100 mg/m². An increase of $t_{0.20}$ by 4 hours i.e. roughly from the median (2.41 hours) to the 95th percentile (6.16 hours) at first course increases the risk by 40% beyond the effect of cumulative dose.

Baseline AAG level was a significant predictor of all the PD endpoints investigated in this study.

Example 2

This example is illustrative of the present invention. It provides information stemming from a study of alpha-1-acid glycoprotein as an independent predictor of response and survival in patients with non-small cell lung cancer treated with docetaxel.

The Patient Pool

The data for this study was prospectively collected from unresectable and metastatic NSCLC patients entered into six Phase II open label, non-randomized studies of docetaxel (Burris H, Eckardt J, Fields S, et al: Phase II trials of Taxotere in patients with non small cell lung cancer. Proc Am Soc Clin Oncol 12: 335, 1993 (abstr 1116); Cerny T, Kaplan S, Pavlidis N, et al: Docetaxel (Taxotere) is active in non-small-cell lung cancer: A phase II trial of the EORTC Early Clinical Trials Group. Br J Cancer 70: 384-387, 1994; Fossella FV, Lee JS, Murphy WK et al: Phase II trial of docetaxel for recurrent or metastatic non-small cell lung cancer. J Clin Oncol 12: 1238-1244, 1994; Francis PA, Rigas JR, Kris MG et al: Phase II trial of docetaxel in patients with Stage III and IV non-small cell lung cancer. J Clin Oncol 12: 1232-1237, 1994; Fossella FV, Lee JS, Shin DM, et al: Phase II study of docetaxel for advanced or metastatic platinum-refractory non-small-cell lung cancer. J Clin Oncol 13: 645-651, 1995; Miller VA, Rigas JR, Francis PA, et al: Phase II trial of a 75 mg/m^2 dose of docetaxel with prednisone premedication for patients with advanced non-small cell lung cancer. Cancer 75: 968-972, 1995). Detailed information and clinical trial results for these studies have been previously reported.

The criteria for eligibility included confirmation of non-small cell lung cancer, one or more bidimensionally measurable lesion, adequate bone marrow (absolute neutrophil count > 2,000/mL), renal (normal creatinine) and hepatic function (total bilirubin < 1.25 x upper limit of normal (ULN), alanine aminotransaminase (ALT) ≤ 2 x ULN). According to the study design, patients may have received prior treatment. The initial docetaxel dose for most patients was 100 mg/m^2 given as a 1-hr infusion every 3 weeks. Dose reduction of twenty-five percent or delay of subsequent courses of therapy was permitted, based on the grade of toxicity observed. These studies were part of the 22 Phase II studies reported in a previous PK/PD analysis of docetaxel (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics

Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998).

M asurement of AAG Levels

AAG levels were determined by a variety of methods, primarily by the Bienvenu et al. laser nephelometry method. (See Bienvenu et al. Clinical Chemistry, 27(5), 721-726 (1981) and Example 3.)

Pharmacokinetic Data

Pharmacokinetic assessment was performed at the first cycle of treatment. The design of the sampling strategy was presented in Example 1 and in detail in Bruno *et al* (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics /Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998). Briefly, the sampling strategy consisted of four different sampling schedules of 3 sampling times which were randomly assigned to patients upon study entry. Docetaxel was assayed in plasma samples using high performance liquid chromatography and UV detection after solid-phase extraction (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992).

From the population pharmacokinetic parameters (Bruno R, Vivier N, Vergniol JC et al: A population pharmacokinetic model for docetaxel (Taxotere®) : Model building and validation. J Pharmacokinet Biopharm 24:153-172, 1996), Bayesian methods were used to estimate each individual's pharmacokinetic parameters from the patient's plasma concentrations (Baille P, Bruno R, Schellens JHM et al: Optimal sampling strategies for Bayesian estimation of docetaxel (Taxotere®) clearance. Clin Cancer Res 3:1535-1538, 1997). The NONMEM computer program was employed for these studies (version IV, level 2.0) (Beal SL, Boeckman AJ, Sheiner LB. NONMEM. User's Guide Part I to VI. University of

California at San Francisco, San Francisco, 1988 - 1992). The PK model used a three-compartment structural model with first-order elimination and the PK parameters considered for this analysis are CL, and AUC.

Clinical Endpoints

The following clinical endpoints were considered for this analysis.

Safety : Febrile neutropenia, infections, grade 3/4 stomatitis, grade 3/4 diarrhea and severe asthenia, reported during the first course of therapy were considered as safety endpoints. These parameters were selected as they typically require dose reduction or treatment delay. Stomatitis and diarrhea were defined and graded using the Common Toxicity Criteria of U.S. National Cancer Institute whereas COSTART classification was used for asthenia. Febrile neutropenia was defined as body temperature > 38°C with concomitant NCI grade 4 neutropenia (neutrophil count < 500/mL) requiring antibiotics and/or hospitalization.

Due to the small number of patients and low incidence of severe adverse events, these safety endpoints were pooled for analysis.

Response rate : The patients were considered to be a responder when they experienced either a partial response (PR) or a complete response (CR) using standard criteria. Patients with minor responses (< 50 % reduction in tumor size), evaluable disease, stable disease and patients with disease progression were considered as non responders. Responses had to be confirmed after a minimum of 4 weeks and were reviewed by an independent panel.

Survival : Survival was calculated from the date of the first infusion to the date of death, last contact for patients lost of follow-up, or a cut-off date for patients alive at the time of closure of the data set.

Data Analysis

Three categories of independent variables thought to affect survival in NSCLC were considered for this analysis. Firstly, docetaxel exposure as assessed by the cumulative dose, or CL and AUC at first course Secondly, the patient characteristics including age, gender, performance status, alpha-1-acid glycoprotein, lactate dehydrogenase, baseline neutrophil count, time from initial diagnosis of NSCLC, number of disease sites, visceral cancer involvement, hepatic metastasis and bone metastasis. Thirdly, the extent of prior treatment reported as prior chemotherapy, number of prior chemotherapy regimens, prior cisplatin, and prior radiotherapy.

A logistic regression was used to relate binary endpoints, such as the incidence of severe adverse events and response rate, to the independent variables, while a Cox regression was used for the survival analysis. Cumulative docetaxel dose was the only time-dependent covariate used in the Cox model. Univariate and multivariate analyses were conducted. The multivariate model involved a stepwise selection of covariates starting from the null model. Significance levels for variable entry or removal at each step in the development of the multivariate model were $p<0.10$ and $p<0.05$, respectively. The median survival was estimated using the Kaplan-Meier method. Analyses were carried out using the SAS software (SAS version 6.12; SAS Institute Inc., Cary, NC).

Discussion of Results

Patient characteristics at baseline

Overall, 189 patients of the 269 NSCLC patients entered in the six Phase II studies of docetaxel (70 %) had pharmacokinetic data available for analysis. Nine patients received 75 mg/m^2 of docetaxel as their initial dose, and all other patients (n=180) received 100 mg/m^2. This analysis was restricted to the patients treated with 100 mg/m^2 of

docetaxel. Among these patients, 143 were evaluable for response, however, the analysis was conducted on the intent-to-treat population of 189 patients. Some models were reassessed on the evaluable patient population as a sensitivity analysis to examine their affects on the predictor outcomes. The patient characteristics are summarized in **Table 7**. Median age for this population of NSCLC patients was 61 years, two thirds were male, 82% of the patients had a WHO performance status of 0 to 1. Most of the patients were chemotherapy naive (71%), and had metastatic disease (77%).

Table 7. Patient characteristics and docetaxel exposure (n = 180)

percentile		Number	%	median	5%-95%
Age (years)				61	43-72
Sex	Male	118	(66)		
	Female	62	(34)		
WHO performance status	0	35	(19)		
	1	113	(63)		
	2	32	(18)		
α1-acid glycoprotein (g/l)				1.42	0.84-2.71
Time from diagnosis (month)				4.7	0.6-39
	>12 month	48	(27)		
<u>Extent of disease</u>					
Number of disease sites	1	48	(27)		
	2	70	(39)		
	3	42	(23)		
	≥4	20	(11)		
Liver metastasis		34	(19)		
<u>Prior treatments</u>					
Chemotherapy		52	(29)		
Number or prior regimen	0	128	(71)		
	1	34	(19)		
	≥2	18	(10)		

percentile	Number % median	5%-95%
Prior platinum	43 (24)	
Radiotherapy	70 (39)	
Docetaxel exposure		
CL (L/h)	35.7	17.8-58.8
AUC (mg.h/mL)	4.98	3.24-9.76

Individual PK parameter estimates

Individual estimates of PK and exposure parameters are given in **Table 7.** In this NSCLC patient population, the median clearance was 35.7 L/h varying from 17.8 L/h to 58.8 L/h (5% to 95% percentile range). This clearance distribution was very similar to that of the larger population of patients with various tumor types with a median of 36.3 L/h (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics /Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998); The observed median AUC was 4.98 mg·mL/h with a 5% to 95% percentile range 3.24 mg·mL/h to 9.76 mg·mL/h.

Severe adverse events

Twenty-five patients (13.9 %) experienced at least one severe adverse event during the (TOX) first cycle of therapy (**Table 8**). Docetaxel exposure as measured by the AUC was the only significant predictor of these adverse events ($p<0.0001$). A high AUC was associated with increased probability of experiencing any of the severe toxicities. Age of the patients had a borderline significant effect ($p = 0.056$), with older patients showing a trend towards a higher probability of experience a severe adverse event.

Tabl 8. Incidence of adverse events at cycle 1

	number	%
Febrile Neutropenia	7	3.9
Infection	8	4.4
Stomatitis (Grade 3, 4)	3	1.7
Diarrhea (Grade 3, 4)	10	5.6
Asthenia (severe)	2	1.1
Endpoint		
TOX*	25	13.9
TOX1**	23	12.8

*patients experienced at least one event

**patients experienced febrile neutropenia or infection or grade 3 diarrhea

Subsets of associated toxicities were also analyzed for their correlative significance. In all subsets, AUC was the only significant predictor of these severe adverse events. In one subset that included febrile neutropenia or infection or diarrhea (TOX1), with 23 adverse events, (12.8%), AAG reached borderline significance (p=0.0505) in addition to AUC.

The odds ratio for the logistic regression models calculated for the relevant covariate changes from the 25th to the 75th percentiles are given in **Table 9**. According to the logistic model, the odds of experiencing a severe adverse reaction was approximately 2 fold greater for a change in AUC from 4.2 to 6.5 mg·h/mL. While, an increase in the AAG from 1.11 to 1.85 g/L resulted in roughly a 50 % reduction in the odds of experiencing one toxicity event from the TOX1 group.

Table 9. Logistic regression models for adverse events at cycle 1

Endpoint	Predictor	p	Odds Ratio*	(95 % CI)
TOX	AUC (4.2 to 6.5 mg.h/mL)	0.0021	1.81	(1.24 - 2.64)
TOX1	AUC (4.2 to 6.5 mg.h/mL)	0.0005	2.37	(1.46 - 3.87)
	AAG (1.11 to 1.85 g/L)	0.0505	0.47	(0.22 - 1.00)

*odds ratio for covariate change from 25th to 75th percentiles

for AUC and for AAG

Response rate

The overall response rate was 29% in both intent-to-treat and evaluable populations. Baseline AAG was the only significant predictor of response rate (p=0.0039) with an odds ratio of 0.44 for a change in AAG from 1.11 to 1.85 g/L. An increase in the baseline AAG level was associated with a 56% decrease in the odds of response (**Table 10**). The response rate was 41.3% (95% CI : 27.0% - 56.8%) for patients with a low AAG (AAG $\leq$ 1.11 g/L, n = 46) and 15.9% (95% CI : 6.7% - 30.1%) for patients with a high AAG (AAG $\geq$ 1.85 g/L, n = 44).

Table 10. logistic regression model for response*

Predictor	p	Odds Ratio** (95 % CI)
AAG (1.11 to 1.85 g/L)	0.0039	0.44 (0.25 - 0.77)

* intent-to-treat population, response rate = 25.0 %

*odds ratio for AAG change from 25th to 75th percentiles

In the univariate analyses, in addition to baseline AAG levels, trends were observed for a lower odds of response in patients with metastatic disease (p=0.054), in patients who received radiotherapy prior to docetaxel treatment (p=0.055), in younger patients (p=0.080) and in patients with a poor performance status (p=0.080). However, when baseline AAG was included in the multivariate analysis, none of these covariates entered the model even at a significance level of p<0.10. Similar findings were very obtained for the patients with evaluable disease.

Survival

The most significant univariate predictors of survival were cumulative dose, baseline AAG and number of sites of disease (p<0.0001). Clearance or AUC, prior radiotherapy, gender and performance status were also significant predictors of survival (p<0.05). The risk of death decreased as the cumulative dose of docetaxel increased. However, an increased risk of death was observed for patients with higher AAG, two or more sites of disease, low CL or high AUC, poor performance status, female gender and for patients having received prior therapy.

Only cumulative dose, AAG and two or more disease sites remained significant in the multivariate analysis (**Tabl 11**). The risk of death decreased by 20 % for each additional cycle of treatment and roughly doubled in patients with a high AAG (1.85 g/L) compared to patients with a low AAG (1.11 g/L) and in patients with two or more sites of disease.

Table 11. Cox regression model for survival*

Predictor	p	Risk Ratio** (95 % CI)
Cumulative dose***(100mg/m²)	< 0.0001	0.82 (0.74 - 0.90)
AAG (1.11 to 1.85 g/L)	< 0.0001	1.76 (1.40 - 2.21)
No disease sites (< 2 to ≥ 2)	0.0049	1.96 (1.23 - 3.12)

* death occurred in 70.5 % of the patients (127 of 180 patients)

** risk ratio for change of covariates given in brackets

*** time-dependent covariate

When baseline AAG was not considered in the stepwise multivariate analysis, it was replaced by performance status (p=0.0053), gender (p=0.025) and prior radiotherapy (p=0.045). Therefore, the pretreatment AAG level appeared to be a more important predictor of survival in NSCLC patients treated with docetaxel than several other known prognostic factors. The median survival (**Table 12** and **Figure 4**) varied from 15.6 months in low AAG patients (AAG ≤ 1.11 g/L, n=46) to 5.5 months in high AAG patients (AAG ≥ 1.85 g/L, n=44). Patients with intermediate AAG values (n=90) had a median survival time of 9.2 months.

Table 12. Survival as a function of alpha-1-acid glycoprotein baseline level

	alpha-1-acid glycoprotein (g/L)			
	≤ 1.11* (n=46)	1.12 - 1.84 (n=90)	≥ 1.85** (n=44)	Log-Rank
median (month)	15.6	9.2	5.5	< 0.0001
95% CI	(11.8-20.0)	(6.4-11.4)	(4.1-7.5)	

* 25% quantile of AAG distribution

** 75% quantile of AAG distribution

Over the last decade performance status has been recognized as the most important predictor of response, and survival in patients with advanced NSCLC (Ginsberg RJ, Vokes EE, Raben A: Non-small cell lung cancer, in De Vita VT, Hellman S, Rosenberg SA (eds): Cancer Principles & Practice of Oncology. Volume 1, Chapter 30, Section 2, 5th Edition, Philadelphia, New York, Lippincott-Raven, 1997, pp 858-911). This study shows that NSCLC patients with a high baseline AAG have a lower response rate (14% compared to 44% in patients with a low AAG) and a markedly shorter survival (median of 5.5 month compared to 15.6 months in patients with a low AAG).

Example 3

This example is representative of methods used to determine AAG levels. 500 μL samples of blood were collected in a polystyrene microtube without anticoagulant by venous puncture. The serum was removed after centrifugation.

For laser nephelometry a Behring Laser Nephelometer module I was used. (Behringwerke, D-3550 Marburg/Lahn, Germany). Samples, standards, and antisera were diluted with sterile isotonic saline solution and 100 μL of 101-fold diluted sample were mixed in a microcuvette with 200 μL of a fivefold diluted anti-orosomucoid antiserum (LN serum anti-orosomucoid (AAG) SAW; Behringwerke). The cuvettes were shaken briefly and allowed to stand for 1 hour at room temperature, and the light scattered by the resulting antigen-antibody complexes was measured (in volts) with the nephelometer. A calibration curve was prepared by use of an 800mg/L standard solution of orosomucoid, diluted to give concentrations of 40, 20, 10, 5, 2.5, and 1.25 mg/L. The blank values (i.e., the light scattered by the empty cuvettes) were negligible (80-150 mV).

Example 4 - This example presents a clinical trial simulation for exploring the safety profile of docetaxel (Taxotere®) in cancer patients.

Docetaxel exposure and alpha-1-acid glycoprotein level (AAG) predict hematological toxicities of docetaxel (Bruno et al., *J. Clin. Oncol.*, 16, 187 (1998)). To assess the impact of increasing doses on the safety profile of docetaxel, in patients with different AAG levels, 100 complete trials were stochastically simulated (ACSL Biomed). In each trial, 600 patients were randomly assigned to groups of either low (L) (≤ 1.11 g/L), intermediate (I) (1.12-1.84 g/L) or high (H) (≥ 1.85 g/L) AAG and received 60, 75, 100 and 125 mg/m^2 of docetaxel intravenously over 1 hour. The simulated median AUC, median incidence of grade 4 neutropenia (GR4) and febrile neutropenia (FEB) were:

Group	Dose (mg/m^2)	60	75	100	125
L	AUC (μg.h/mL)	2.7	3.4	4.5	5.6
	GR4 (%)	68.9	73.4	79.9	84.5
	FEB (%)	4.3	5.3	7.3	10.1

Group	Dos (mg/m^2)	60	75	100	125
	AUC (μg.h/mL)	3.0	3.8	5.0	6.3
	GR4 (%)	41.1	46.5	56.6	66.3
I	FEB (%)	2.3	2.9	4.4	6.5
	AUC (μg.h/mL)	3.8	4.7	6.3	7.9
	GR4 (%)	13.3	17.5	26.1	35.7
H	FEB (%)	1.0	1.0	1.9	3.6

The results demonstrate that the dose response of docetaxel is markedly influenced by AAG and these results provide insights for the design of future trials.

Example 5 - This examples summarizes studies on Alpha-1-acid glycoprotein as an independent predictor of efficacy and survival in NSCLC patients treated with docetaxel (Taxotere®).

Baseline alpha-1-acid glycoprotein (AAG) and docetaxel docetaxel clearance (and/or exposure) were previously found to be independent predictors of docetaxel safety (all tumor types combined) and of time to progression (TTP) in NSCLC (Bruno et al., *J. Clin. Oncol.* (Vol. 16, No. 1, 1998, pp. 187-196)). The predictors of treatment outcome and survival of advanced NSCLC patients entered in 4 Phase II studies (n=180) of docetaxel (100 mg/m^2) were investigated using logistic and Cox multivariate regressions. Univariate analysis showed that compared to patients with high AAG (≥ 1.92 g/L (75 percentile)), patients with low AAG (≤ 1.09 g/L (25 percentile)) experienced more side effects (e.g. febrile neutropenia: 19% vs. 2.3%, p=0.02) but had a higher response rate (44% vs. 14%, p=0.002), a longer TTP (18 vs. 9.7 weeks, p=0.006) and a much longer survival: 16 months compared to 5.2 months (p<0.0001). In multivariate models, in addition to TTP (Bruno et al., *supra*), AAG was an independent prognostic factor for the incidence of severe side effects at first cycle (p=0.006 with an interaction with clearance), for response rate (odds ratio for non response in high AAG

patients: 5.5, p=0.006), and for survival (p<0.0001). In conclusion, low AAG is independently associated with better efficacy and longer survival in advanced NSCLC treated with docetaxel.

CLAIMS

1. A method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein comprising: (A) observing the patient's level of alpha-1-acid glycoprotein; (B) evaluating said level to determine the dosage of the taxoid to administer to the patient by comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said evaluation, recommending the dosage of the taxoid to administer to the patient.

2. The method of claim 1 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

3. The method of claim 1 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

4. The method of claim 3 wherein said cancer is non-small cell lung cancer.

5. The method of claim 1 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

6. A method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid comprising: (A) observing the patient's alpha-1-acid glycoprotein level; (B) comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a

population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said comparison, assessing the effect of continued treatment of the patient with respect to the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient.

7. The method of claim 6 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

8. The method of claim 6 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

9. The method of claim 8 wherein said cancer is non-small cell lung cancer.

10. The method of claim 6 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

11. The method of claim 6 wherein said patient is being treated with a dosage of about 55 to about 200 mg/m^2 of taxoid.

12. The method of claim 6 wherein said patient is being treated with about 55 to about 125 mg/m^2 docetaxel.

13. The method of claim 6 wherein said patient is being treated with about 135 to about 175 mg/m^2 paclitaxel.

14. A method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid comprising: (A) observing the patient's alpha-1-acid glycoprotein (AAG) level; (B) comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said comparison recommending the dosage of said taxoid to administer to said patient to reduce the incidence or severity of side effects that the patient may experience during treatment with said taxoid.

15. The method of claim 14 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

16. The method of claim 14 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

17. The method of claim 16 wherein said cancer is non-small cell lung cancer.

18. The method of claim 14 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

19. The method of claim 14 wherein said population of patients is being treated with a dosage of about 55 to about 200 mg/m^2 of said taxoid.

20. The method of claim 14 wherein said patient is being treated with about 55 to about 125 mg/m^2 of docetaxel.

21. The method of claim 14 wherein said patient is being treated with about 135 to about 175 mg/m^2 paclitaxel.

22. The method of claim 14 wherein the side effects are selected from the group consisting of neutropenia, infection, diarrhea, infusion-related hypersensitivity reactions, allopenia, neurotoxicity, mucositis, stomatitis, severe asthenia, fluid retention and myalgias.

23. The method of claim 22 wherein said side effect is neutropenia.

24. The method of claim 23 wherein said neutropenia is febrile neutropenia.

25. The method of claim 14 wherein said taxoid is docetaxel and said dosage is recommended to be less than about 100 mg/m^2.

26. The method of claim 14 wherein said taxoid is paclitaxel and said dosage is recommended to be less than about 175 mg/m^2.

27. The method of claim 14 wherein the recommended dosage is about 5 to about 35% below said common dosage.

28. The method of claim 14 wherein the recommended dosage is reduced by about 10 to about 30% below said common dosage.

29. The method of claim 14 wherein the recommended dosage is reduced to about 15 to about 27% below said common dosage.

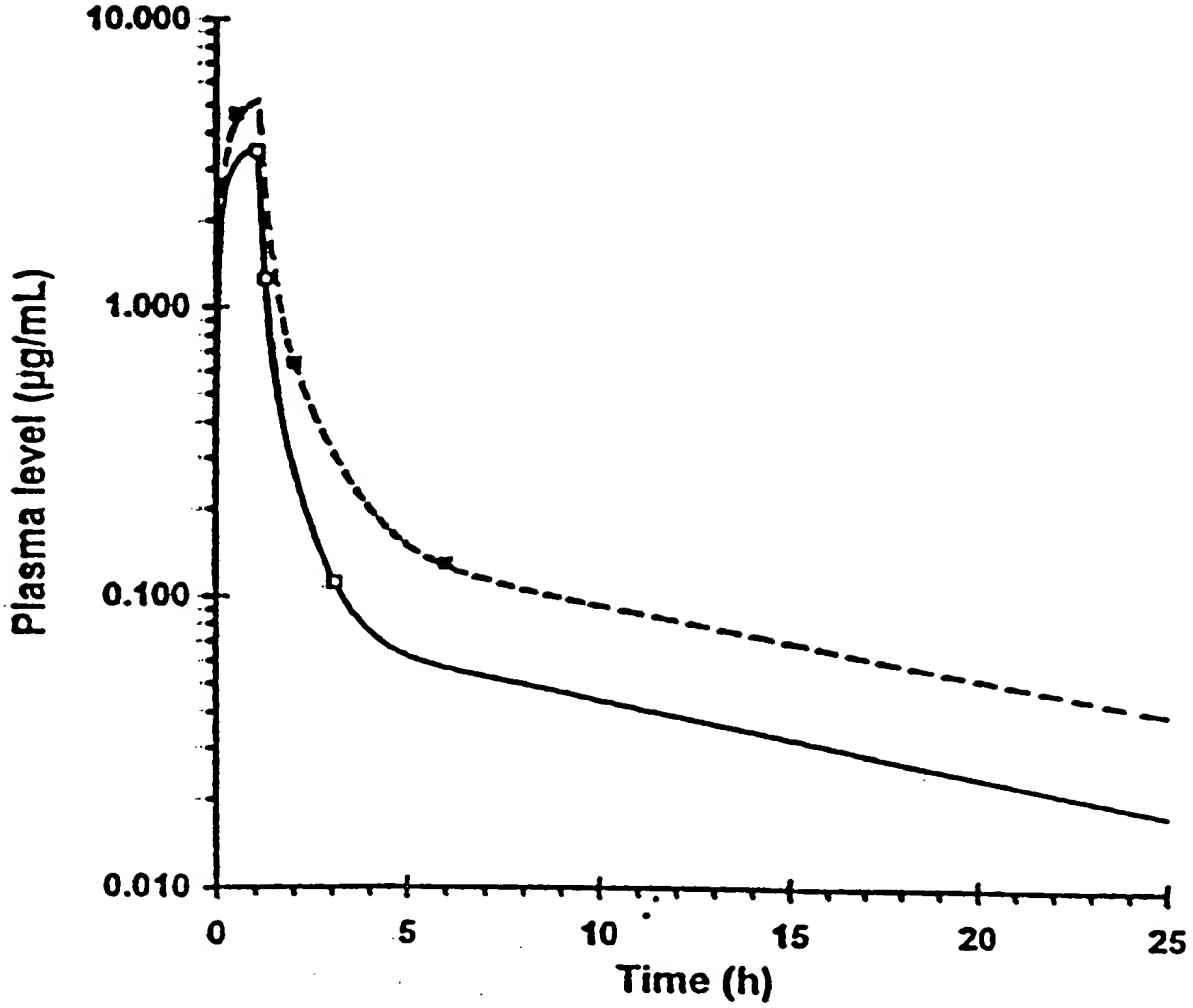

Fig 1. Docetaxel PK profile in representative patient with normal liver function (□) and patient with elevated hepatic enzymes (—■—). Lines denote model predictions after Bayesian estimation.

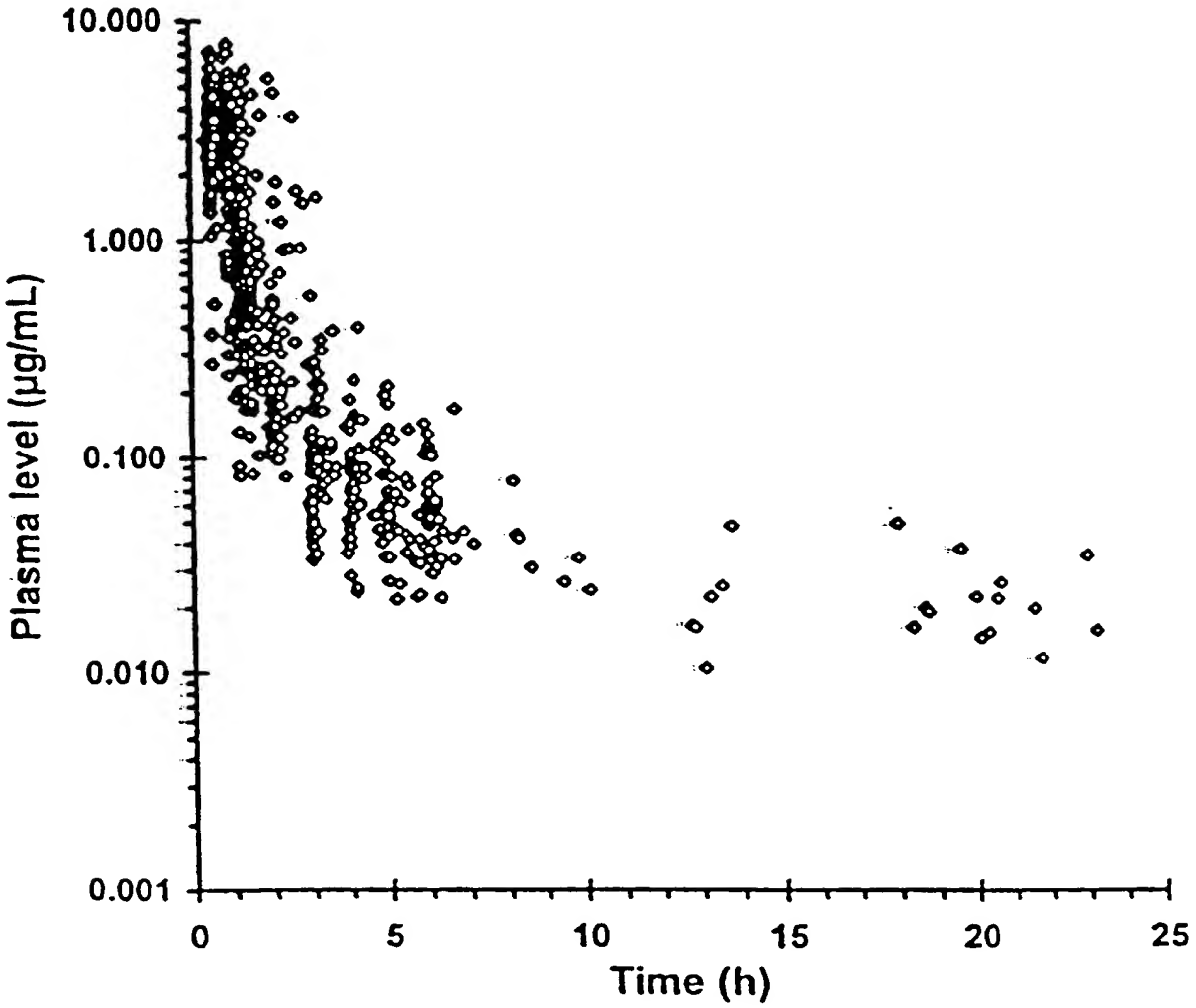

Fig 2. Docetaxel population PK profile in a subset of 254 patients.

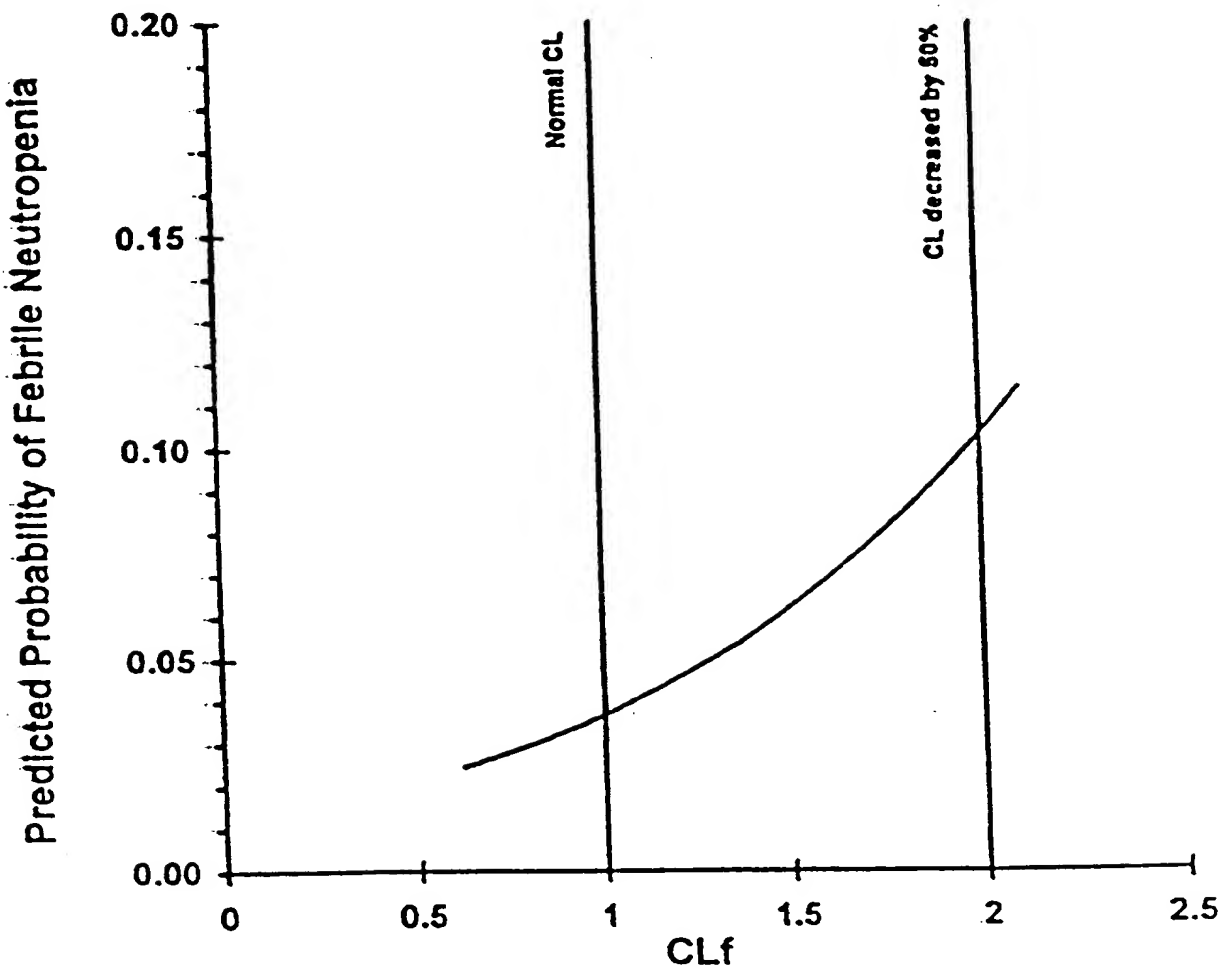

Fig 3. Model-predicted probability of febrile neutropenia as a function of CLf for a patient with median AAG. Reference vertical lines denote normal CL (CLf = 1) and 50% reduced CL (CLf = 2).

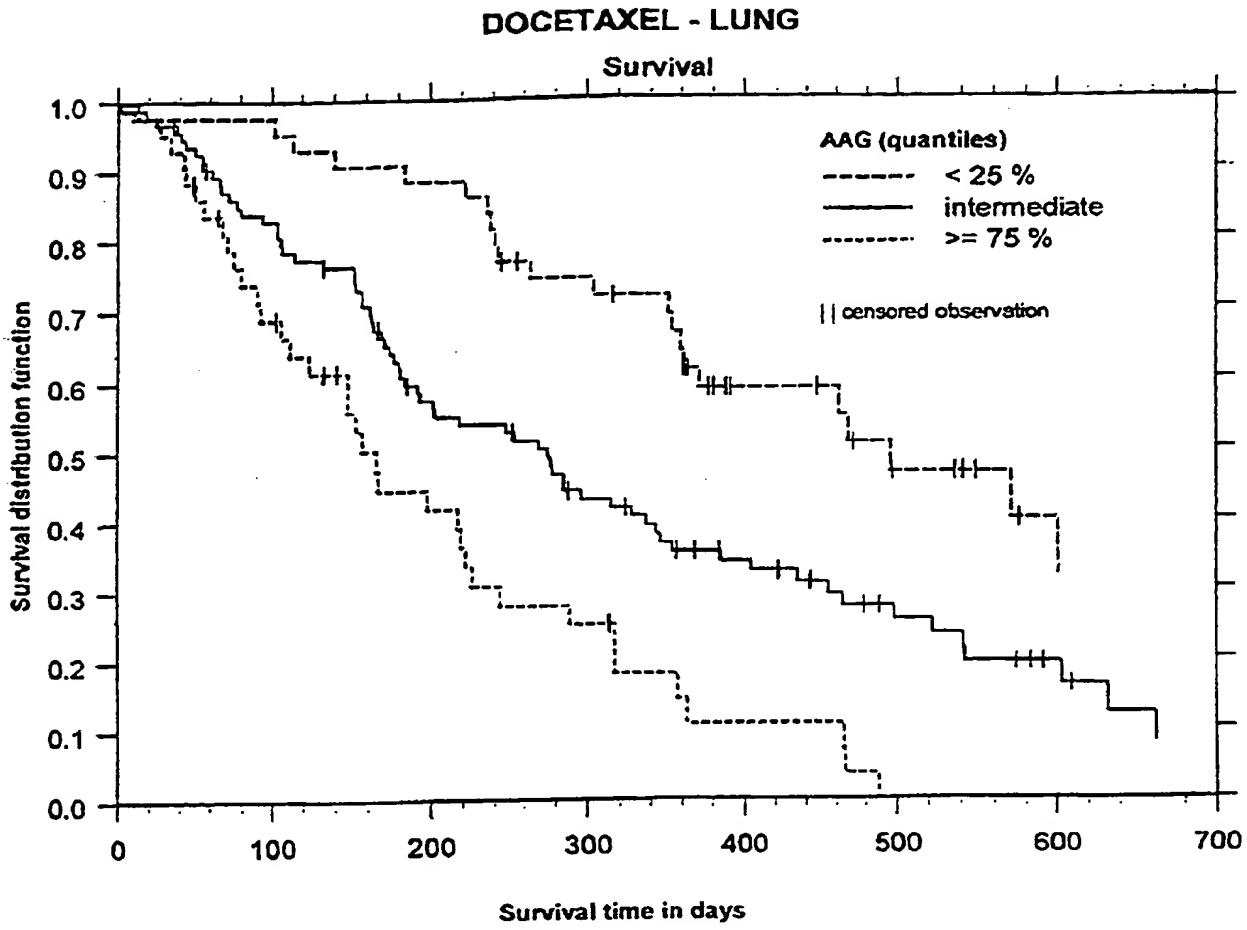

Figure 4 : survival curves in NSCLC patients with low (≤ 1.11 g/L, —), intermediate (1.12 to 1.84 g/L, ----) and high (≥ 1.85, -•-•-) baseline AAG (/censored observation)

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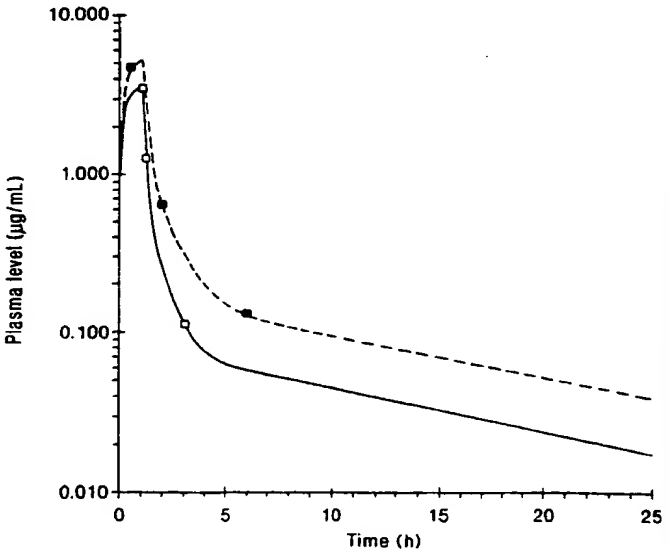
(54) Title: PREDICTIVE METHODS BASED ON ALPHA–1–ACID GLYCOPROTEIN LEVELS

(57) Abstract

A method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein comprising observing the patient's level of alpha-1-acid glycoprotein, evaluating said level to determine the dosage of the taxoid to administer to the patient by comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said evaluation, recommending the dosage of the taxoid to administer to the patient. Also, a method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid comprising observing the patient's alpha-1-acid glycoprotein level, comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison, assessing the effect of continued treatment of the patient with respect to the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient. Also, a method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid comprising observing the patient's alpha-1-acid glycoprotein (AAG) level, comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison recommending the dosage of said taxoid to administer to said patient to reduce the incidence or severity of side effects that the patient may experience during treatment with said taxoid.



Docetaxel PK profile in representative patient with normal liver function (o) and patient with elevated hepatic enzymes (---•---). Lines denote model predictions after Bayesian estimation.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

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B. FIELDS SEARCHED

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IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	VEYRAT-FOLLET C. ET AL.: "Application of clinical trial simulation in exploring the safety profile of docetaxel (D) in cancer patients" CLINICAL PHARMACOLOGY AND THERAPEUTICS, vol. 65, no. 2, February 1999 (1999-02), page 198 XP000908903 abstract ---	1-13
X	URIEN S. ET AL.: "Docetaxel serum protein binding with high affinity to alphal-acid glycoprotein" INVESTIGATIONAL NEW DRUGS, vol. 14, 1996, pages 147-151, XP000908900 abstract page 150, column 1, paragraph 2 -page 151, column 1, paragraph 1 --- -/--	1-13

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2000	29/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pellegrini, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BRUNO R. ET AL.: "A population pharmacokinetic model for docetaxel (Taxotere): model building and correlation" JOURNAL OF PHARMACOKINETICS AND BIOPHARMACEUTICS, vol. 24, no. 2, - 1996 pages 153-172, XP000908881 cited in the application abstract page 169, paragraph 5 page 170, paragraph 5 ---	1-13
X	BRUNO R. ET AL.: "Population pharmacokintetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer" J. CLIN. ONCOL., vol. 16, no. 1, January 1998 (1998-01), pages 187-196, XP000910385 cited in the application abstract page 191, column 1, line 18-20 ---	1-13
X	GANZ P.A. ET AL.: "Monitoring the therapy of lung cancer with alpha-1-acid glycoprotein" CANCER RESEARCH, vol. 44, 1984, pages 5415-5421, XP000909045 abstract page 5416, column 1, paragraph 3 -page 5416, column 2, paragraph 1 ---	1-13
X	GANZ P.A. ET AL.: "Evaluation of a radioimmunoassay for alpha-1-acid glycoprotein to monitor therapy of cancer patients" JOURNAL NATIONAL CANCER INSTITUTE (JNCI), vol. 71, no. 1, 1983, pages 25-30, XP000909046 cited in the application abstract page 26, column 1, paragraph 2 ---	1-13
X	BIENVENU J. ET AL.: "Laser nephelometry of orosomucoid in serum of newborns: reference intervals and relation to bacterial infections" CLIN. CHEM., vol. 27, no. 5, 1981, pages 721-726, XP002139731 cited in the application page 721, column 2, paragraph 5 -page 722, column 1, paragraph 2 --- -/--	1-13

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KREMMER T. ET AL.: "Determination and analysis of human serum alpha-1-acid glycoprotein by liquid chromatographic methods" J. LIQUID CHROMATOGRAPHY, vol. 18, no. 6, 1995, pages 1207-1218, XP000909051 page 1209, paragraph 1 -page 1210, paragraph 2 -----	1-13

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 14-29

Subject-matter for which protection os sought in claims 14-29 is excluded from patentability because it relates to a method for treatment of the human or animal body by therapy (Rule 39.1.(iv) PCT). However, a search has been performed on analytical tests for determining alpha-1-acid glycoprotein levels and on the correlation between alpha-1-acid glycoprotein levels and the pharmacokinetics/pharmacodynamics of taxoids.

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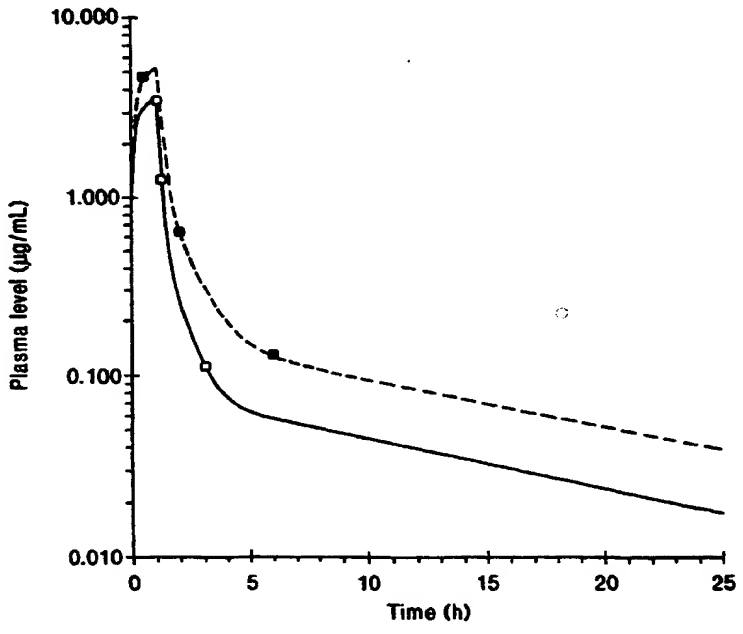
[Continued on next page]

(54) Title: PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS



(57) Abstract: A method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein comprising observing the patient's level of alpha-1-acid glycoprotein, evaluating said level to determine the dosage of the taxoid to administer to the patient by comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said evaluation, recommending the dosage of the taxoid to administer to the patient. Also, a method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid comprising observing the patient's alpha-1-acid glycoprotein level, comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison, assessing the effect of continued treatment of the patient with respect to the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient. Also, a method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid comprising observing the patient's alpha-1-acid glycoprotein (AAG) level, comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage and based on said comparison recommending the dosage of said taxoid to administer to said patient to reduce the incidence or severity of side effects that the patient may experience during treatment with said taxoid.

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PREDICTIVE METHODS BASED ON ALPHA-1-ACID GLYCOPROTEIN LEVELS

Cross-Reference to Related Application

This application claims the benefit of U.S. Provisional Application No. 60/114,520, filed December 30, 1998.

Field of the Invention

This invention relates to methods which are useful in the treatment of cancer with a pharmaceutical that is a member of the family of anti-neoplastic agents known as taxoids.

The family of anti-neoplastic agents known as taxoids are based on natural and modified compounds that have the taxone skeleton isolated from the yew tree (Taxaceae). Two particularly effective taxoids are paclitaxel, which is a natural product isolated from the Pacific yew (Taxus brevifolia), and docetaxel, which is a semisynthetic product derived from the needles of the European yew (Taxus baccata). The activities of these agents have been demonstrated in a wide variety of cancers, including breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. Other taxoids are being developed for cancer treatment also.

The taxoids appear to show a common mechanism of action based on promoting the assembly and inhibiting the disassembly of microtubules. This causes disruption of the microtubular network that is required for mitotic and interphase cellular functions thereby disrupting cell proliferation.

For a patient being treated with a taxoid, it is desirable to be able to predict the efficacy of the treatment and/or a suitable dosage level to administer to the patient. Efficacy can be characterized by the response to taxoid treatment and the survival of the patient. Suitable dosage levels relate to reducing or avoiding undesirable side effects that might be experienced by the patient while maintaining efficacy.

Reported Developments

Descriptions of clinical studies relating to the efficacy and side effects resulting from the clinically available taxoids, paclitaxel and docetaxel (TAXOTERE®) are provided in The Physicians Desk Reference, 52nd edition (1998) p762-766 (paclitaxel) and 2385-2389(TAXOTERE®). An extensive review of the clinical and preclinical profiles of docetaxel are presented in Cortes, J.E., and Pazdur, R.J., Clin. Oncol. 13(10) 2643-2655,(1995). An extensive review of the chemotherapy trials treating advanced breast cancer using the taxoids is provided in Clemens, M. et al., Eur. J. Cancer 33(13) 2183-21939(1997). A review of the pharmacokinetic parameters of paclitaxel and docetaxel and side effects experienced with the use of these taxoids is provided in Verweij, J. et al Ann. Oncol 5(6) p495-503 (1994).

Summary of the Invention

In accordance with the present invention, there is provided a method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein (AAG). The method includes observing the patient's level of AAG,

evaluating the AAG level to determine the dosage of a taxoid to administer to the patient by comparing the AAG level to a predetermined AAG level from a population of patients that have the same type of cancer and who are being treated with the taxoid at a common dosage, and, based on this evaluation, recommending the dosage of the taxoid to administer to the patient.

The taxoids can be, for example, docetaxel or paclitaxel. Examples of the type of cancers that can be treated include breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. An example of a preferred of a preferred embodiment of the present invention involves the treatment of non-small cell lung cancer.

Another aspect of the present invention is the provision of a method for assessing the effect of treatment of a patient who has cancer and who is being treated with a taxoid. This method includes observing the patient`s AAG level, comparing the AAG level to a predetermined AAG level derived from a population of patients having the same cancer and being treated with the taxoid at a common dosage, and, based on this comparison, assessing the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient.

In preferred embodiments, the patient is treated with a dosage of about 55 mg/m^2 to about 200mg/m^2 of the taxoid. In especially preferred embodiments of the invention, the patient is treated with about 55 to about 125 mg/m^2 of docetaxel or about 135 to about 175 mg/m^2 of paclitaxel.

Still another aspect of the present invention is the provision of a method for reducing the side effects experienced by a patient who has cancer and who is to be tr ated with a taxoid. This method includes observing the patient's AAG level, comparing the AAG level to a predetermined AAG level derived from a population of patients

having the same cancer and treated with the taxoid at a common dosage, and, based on this comparison, recommending the dosage of the taxoid to administer to the patient to reduce the incidence or severity of side effects that the patient may experience during treatment with the taxoid.

Examples of side effects include neutropenia, infection, diarrhea, infusion related hypersensitivity reactions, allopenia, neurotoxicity, mucositis, stomatitis, sever asthenia and myalgia. Neutropenias include febrile neutropenia.

Brief Description of the Drawings

Figure 1 is a pharmacokinetic profile of the taxoid docetaxel in a representative patient with normal liver function (□) and a patient with elevated hepatic enzymes (■). Lines denote model predictions after Bayesian estimation.

Figure 2 is a docetaxel pharmacokinetic profile in a subset of 254 patients.

Figure 3 is a model-predicted probability of febrile neutropenia as a function of CLf for a patient with median AAG. Reference vertical lines denote normal CL (CLf=1) and 50% reduced CL (CLf=2).

Figure 4 shows survival curves in NSCLC patients with low (≤1.11 g/L, --), intermediate (1.12 to 1.84 g/L,……), and high (≥1.85,-·-·-) base line AAG (/censored observation).

Detailed Description of the Invention

In connection with the development of this invention, it has been found that, for a cancer patient being treated with a taxoid, the patient's level of alpha-1-acid glycoprotein can be used to predict response to treatment, survivability, and side effects.

For background purposes there is set forth hereafter information relating to the taxoids and alpha-1-acid

glycoprotein(AAG). Following this information, methods for measuring AAG levels are described and the relationships between AAG levels and response to treatment, survivability, and side effects are discussed. With regard to side effects, methods for reducing the possibility of side effects by measuring a patient's AAG level prior to or during taxoid treatment and adjusting the dosage of the taxoid are discussed.

Taxoids

The present invention relates to treatment methods utilizing taxoids. A variety of taxoids may be used in the practice of the present invention. "Taxoid" as used herein refers to anti-neoplastic agents based on natural and modified compounds that have the taxane skeleton isolated from the yew tree. Preferred taxoids used in the practice of the invention are paclitaxel and docetaxel. Paclitaxel promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. Docetaxel binds free tubulin and promotes assembly of microtubules while simultaneously inhibiting the disassembly of the microtubules. This results in the stabilization of microtubules and inhibition of mitosis. The use of docetaxel is particularly preferred in the practice of the invention.

Paclitaxel has the chemical formula $C_{47}H_{51}NO_{14}$ and has a molecular weight of 853.9. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine (*Physician's Desk Reference, supra*)).

Docetaxel has the chemical formula $C_{43}H_{53}NO_{14}\cdot 3H_2O$ and has a molecular weight of 861.9. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-phenylisoserine,N-*tert*-butyl ester,13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetat 2-benzoate, trihydrate (see *Physician's Desk Reference, supra*).

In the practice of the present invention, any cancer that responds to treatment with the taxoids may be treated using the methods of the present invention. The taxoids are known to have activity against a variety of cancers, including, for example, breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas and soft tissue sarcomas. The taxoids have demonstrated activity against other types of cancers when used alone or in conjunction with other anti-neoplastic agents. The present invention is particularly well suited to patients undergoing treatment for non-small cell lung cancer (NSCLC).

Alpha-1-Acid Glycoprotein

There follows hereafter a description of the biochemical and genetic characteristics of alpha-1-acid glycoprotein (AAG) and a description of its function in the body. This is followed by a description of methods for measuring AAG levels and a discussion of the significance of AAG levels found in patients treated with a taxoid.

AAG Characteristics and Function

Alpha-1-acid glycoprotein (AAG), also called orosomucoid, is found in the seromucoid fraction of human blood plasma. Northern blot analysis of RNA extracted from a variety of tissues demonstrates preferential expression of AAG in the liver. The complete amino acid sequence of AAG is known (Schmid, K. et al., *Biochemistry*, 12, 2711-2724 (1973)) and the carbohydrate moiety has been also identified (Schmid, K. et al., *Prog. Clin. Biol. Res.*, 300, 7-2 (1989)). AAG consists of a single polypeptide chain of 183 amino acids with 21 substitutions possible having a molecular weight of approximately 21 kDa. Within the protein backbone there are five N-glycosylation sites for the attachment of oligosaccharides. There are believed to be at least seven alleles coding for AAG (Yuasa, I. et al., *Hum. Genet.*, 77, 255-258 (1987); Umetsu, K. et al., *Electrophoresis*, 9, 224-226 (1988)). Three phenotypes hav been identified with autosomal co-dominant transmission. The variant forms result from amino acid substitutions. The structure and expression

of the genes coding for AAG have been described and the AAG locus has been mapped to the distal portion of the long arm of chromosome 9 (Dente, L. et al., Embo J., 6, 2289-2296 (1987); Eiberg, H. et al, *Clin. Genet.*, 23, 150-154 (1983)).

The normal function of AAG in the body is not completely understood. Based on *in vitro* studies, AAG may be involved in coagulation, phagocytosis, graft rejection, and wound healing. A review of the biological activities of AAG may be found in Kremer et al.(*Pharm. Rev.*, 40(1), 1-47 (1988)).

AAG is classified as an "acute phase protein" because the concentration of AAG increases following inflammatory stimuli. AAG synthesis also increases several fold during an acute phase response (Ricca et al., *J. Biol. Chem.*, 256, 11199-11202 (1981); Koj et al., *Biochem J.*, 206, 545-553 (1982); Koj et al., *Biochem J.*, 224, 505-514 (1984)). The major inducers of AAG synthesis are the cytokines interleukin-1 (IL-1) and interleukin-6 (IL-6), which act additively to induce transcription of the AAG gene.

Increased levels of AAG due to the acute response have been identified in cancer patients and it has been found that elevated levels of AAG can effect the efficacy of a variety of drugs.

AAG has long been known to bind drugs in plasma. AAG demonstrates high binding affinity for basic drugs (with pK values of 8 or higher). In addition, acidic and neutral drugs have also been shown to bind AAG. For an extensive review of the drug binding activity of AAG, see Kremer et al., (*Pharm. Rev.*, 40(1), 1-47 (1988)).

It is known also that the taxoids are bound by AAG. *In vitro* studies have demonstrated that up to 98% of the taxoid docetaxel is bound by plasma proteins, including AAG (see *Physician's Desk Reference, supra*). Similarly, *in vitro* studies of the binding of paclitaxel to serum proteins at paclitaxel concentrations from 0.1 to 50 μg/ml indicate that

89 to 98% of the paclitaxel is bound. Accordingly, it is expected that AAG has the ability to bind taxoids *in vivo*. Due to this binding interaction, the level of AAG in a patient's plasma becomes significant because, as more AAG is available, a greater percentage of an administered taxoid may be bound.

The present invention involves the relationship between AAG levels in a patient who is treated with a taxoid and response to treatment, survival, and side effects. Accordingly, the present invention involves the measurement of a patient's AAG level.

Methods for Measuring AAG Levels

A variety of bodily fluids and tissues may be used to evaluate a patient's AAG level in connection with the practice of the present invention. Methods of obtaining samples of bodily fluids and tissues are well known in the art. Blood plasma is the preferred bodily fluid used to determine AAG levels in the practice of the present invention. Guidance on obtaining blood samples in order to determine AAG levels may be found in Bienvenu et al., *Clinical Chemistry*, Vol. 27, No. 5, 1981, and in Ganz et al., *JNCI*, Vol. 71, No. 1, July 1983. In preferred embodiments utilizing blood plasma to determine AAG levels, a suitable volume of blood, for example, about 0.5 to about 0.2 ml is taken from a patient, preferably by venipuncture, and is centrifuged under sufficient conditions to isolate the plasma fraction for analysis of the AAG level.

A variety of methods known in the art can be used to determine a patient's AAG level. These methods include methods known for isolation and quantification of a protein. Suitable techniques for isolating and quantifying a protein may be found in a variety of sources, including e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, volumes I and II

(D.N. Glover ed. 1985); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994). Methods used to quantify AAG preferably utilize antibodies. A general overview of immunoassays is provided in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Pubs., NY (1988). Monoclonal or polyclonal antibodies specific for AAG can be used in immunoassays to quantify AAG. Monoclonal antibodies against AAG may be obtained from commercial sources, such as ICN Pharmaceuticals Inc. (Catalog No. 692011) or prepared using techniques known in the art. To produce monoclonal antibodies that bind AAG, hybridomas producing anti-AAG antibodies can be prepared and selected for as described in the literature. For example, mice (i.e., balb/c mice) can be immunized with AAG by intraperitoneal injection. After sufficient time has passed to allow for an immune response, the mice can be sacrificed and the spleen cells obtained and fused with myeloma cells using techniques well known in the art. The resulting hybridomas are then grown in a selective medium, and the surviving cells grown in such medium using limiting dilution conditions. After cloning and recloning, hybridomas can be isolated that secrete antibodies (for example, of the IgG or IgM class) directed against AAG. Immunoassays which can be used to quantitate AAG may include ELISA, competitive immunoassays, radioimmunoassays, indirect immunofluorescent assays and the like. Preferred methods for quantification of AAG include, but are not limited to rocket immunoabsorbant assays (Dewey et al., *J. Immunol.*, 144, 4392-8 (1990); radioimmunoassays (Ganz et al., *JNCI*, 71(1), 25 (July 1983); laser nephelometry (Bienvenu et al., *Clinical Chemistry*, 27(5), 721-726 (1981); and immunoassay in a Cobas Bio centrifugal analyzer (Verme et al., *Clinical Chemistry*, 34(1), 2316-2320 (1988)).

A highly preferred method for determining a patient's AAG level utilizes laser nephelometry as described in Bienvenu et al., *Clinical Chemistry*, 27(5), 721-726 (1981). In this method, a blood sample (0.4 ml to 0.2 ml) is collected by venous puncture, and the serum is removed after

centrifugation. A Behring Laser Nephelometer module I (Behringwerke, D-3550 Marburg/Lahn, Germany) is utilized for taking measurements. Samples, standards, and antisera are diluted with sterile isotonic saline solution and 100 μL of 101-fold diluted sample is mixed in a microcuvette with 200 μL of a fivefold diluted anti-orosomucoid antiserum (LN serum anti-orosomucoid (AAG) SAW; Behringwerke (or other commercially available or prepared antibody)). The cuvettes are shaken briefly and allowed to stand for 1 hour at room temperature, and the light scattered by the resulting antigen-antibody complexes is measured (in volts) with the nephelometer. A calibration curve is prepared by use of an 800mg/L standard solution of AAG, diluted to give concentrations of 40, 20, 10, 5, 2.5, and 1.25 mg/L and the concentration of the AAG in the sample is calculated based on its light scattering relative to the known standards.

The present invention includes within its scope situations in which a patient's AAG level is determined by a third party not involved in the predictive aspects of the present invention.

Significance of AAG Levels

The present invention is based in part on the discovery that, for cancer patients being treated with a taxoid, the patient's AAG level is a prognostic factor which allows predictions to be made regarding response to treatment, survival, and side effects. An explanation follows respecting the significance of AAG levels which are higher or lower than the norm or that fluctuate during treatment.

Typically blood or other body fluid samples are taken after a cancer has been diagnosed and during taxoid treatment. Samples may be taken at any point prior to or during the course of treatment. Blood samples obtained prior to manifestation of cancer may be useful in determination of a baseline AAG level in the absence of disease.

The range of AAG in normal individuals is from about 0.36 g/L to about 1.46 g/L. The level of AAG is often elevated in pathological states such as liver cirrhosis, renal disease and cancer. Individuals with cancer may have elevated AAG levels. For example, a study of patients (n=180) having NSCLC had levels of AAG ranging from about 0.84 to about 2.71 g/L, with a median of 1.42 g/L. Approximately half of the patients had an AAG level exceeding the maximum AAG level (1.46 g/L) seen in healthy subjects.

Methods known in the art can be used to evaluate and classify ranges of AAG concentrations in "taxoid-treated" patients with various types of cancer. A sample population of patients having a particular type of cancer who are being treated with a particular taxoid at a common dosage may be studied to quantify the relationship between AAG levels and response to treatment, survival and side effects. The term common dosage refers to a population all of which are receiving the same dosage of a taxoid, for example, a dosage of about 100 mg/m^2. Given a common dosage, patients within a population may receive differing absolute amounts of a taxoid depending on their size. The dosage of a taxoid being administered to a population will depend on the type of cancer and the taxoid being used. Generally speaking, it is believed that the taxoid dosage will fall within the range of about 55 to about 200 mg/m^2, but may be higher or lower, as conditions warrant. A typical dosage range will usually be about 75 mg/m^2 to about 175 mg/m^2. For a given type of cancer, the range of AAG concentrations in the population may be obtained and defined as high, intermediate, and low, using data from the population of patients and standard statistical methods. The AAG concentration ranges in the population constitute "predetermined" AAG levels which are then used for comparison and evaluation of an individual patient's AAG level. For example, for a population of patients, the 25% quantile of the AAG distribution in the population can be classified as the low level and the 75% quantile can be classified as the high level, with the >25% quantile to <75% quantile being classified as intermediate level.

Accordingly, in the practice of the present invention, the AAG concentration is determined using any of the assays described above, preferably the Bienvenu et al. Laser Nephelometry assay. Once the AAG level is known, the patient may be classified as having a high, intermediate, or low AAG level according to the quantile level into which the patient's AAG level falls. After the patient's AAG level has been classified, this level may be compared with observations on the relationship between AAG level and response to treatment, survival, and side effects for a patient population having the same type of cancer and being treated with the same type of taxoid.

Response to Treatment

The term "response to treatment" refers to whether a patient responds to treatment according to the standard criteria for partial response (PR=50% reduction in tumor) and complete response (CR= a complete reduction in tumor) as defined by the National Cancer Institute. Non-response is defined as patients with minor responses (< 50 % reduction in tumor size) evaluable disease, stable disease and patients with disease progression.

The present invention provides methods for assessing the effect of treatment of a given cancer with a given taxoid based on observing the patient's AAG level, comparing this level to a predetermined AAG level derived from a population of patients having the same type of cancer and being treated with the same taxoid at a common dosage, and assessing the effect of continued treatment.

The chances of a patient responding to treatment with a taxoid relates to the patient's AAG level. It has been found that there is a significant increase in the chance of a patient responding to taxoid treatment for a patient who exhibits a low AAG level. In general, if a patient has a low AAG level there is an increased chanc of respons to taxoid treatment relative to the chance of response for a patient with high AAG levels.

Accordingly, a blood sample can be obtained from a patient and the observed AAG level classified as high or low according to the guidance provided hereinabove. The AAG level is evaluated in order to consider recommending an adjustment in the taxoid dosage and/or supplementing treatment with additional chemotherapeutic, surgical or radiation treatments to increase the chance of response to treatment. Based on the AAG level, the patient can be classified as having an increased chance of response if the patient has a low AAG concentration. For a patient with a high AAG level, the patient's response may be considered to be reduced relative to patients having low AAG concentrations.

If a quantitative characterization of the relationship between AAG and response rate is desired for a particular type of cancer, one of skill in the art can readily obtain blood samples from a population of patients having a given type of cancer and follow the guidance provided in the Examples below to further define the correlation between AAG levels and response to treatment with a taxoid.

Survival

The term "survival" is defined as the length of the patient's life from the time of the first infusion of a taxoid dosage to the date of death. The present invention provides methods of assessing the effect of treatment as it relates to survival for a patient who has cancer and who is being treated with a taxoid. The method involves observing an individual patient's AAG level, classifying the AAG level as low, intermediate or high AAG compared to predetermined AAG levels in a patient population having the same type of cancer under treatment with the same taxoid at a common dosage and assessing the effect of continued treatment in order to predict the patient's survival. The AAG level is evaluated in view of a population of patients having the same type of cancer to consider recommending an adjustm nt in the taxoid dosage and/or supplementing the treatment with additional chemotherapeutic, surgical or radiation treatment

to prolong survival. In accordance with the present invention, it has been found that patients having low AAG levels will be expected to survive longer than patients with high AAG levels.

If a quantitative description of the relationship between AAG level and survival is desired for a particular type of cancer, one of skill in the art may obtain blood samples from a population of patients having that type of cancer and being treated with the same taxoid at a common dosage and may follow the protocol presented in the Examples below to further define the relationship between AAG level and survival.

In addition to survival, the methods of the present invention are also useful in predicting time to progression. Time to progression is calculated from the first administration of the taxoid to the date of progression as discussed in the examples below. Studies of patients with NSCLC demonstrated that patients with low AAG levels ($\leq$ 1.09 g/L) had a longer time to progression (18 weeks) versus 9.7 weeks for patients with high AAG levels ($\geq$ 1.92 g/L). Accordingly, the methods described above for survival may also be used with regard to time to progression.

Side Effects

The term "side effects" refers to adverse effects produced by a drug such as a taxoid, especially on a tissue or organ system other than the one sought to be treated with the drug. Use of the taxoids can result in a variety of side effects, including, for example, neutropenia, infusion-related hypersensitivity reactions, allopecia, neurotoxicity, mucositis, infections, stomatitis, diarrhea, severe asthenia, fluid retention and myalgias.

The nature and severity of the side effects due to the use of a given taxoid will depend on a variety of factors, including the specific taxoid used, the dosage, the overall

dosing regimen, the presence of other drugs, and factors relating to the patient's physiological state.

The side effects specific to paclitaxel and taxotere are well documented (see *Physician's Desk Reference, supra*, Cortes and Pazdur, *Journal of Clinical Oncology*, vol. 13, No. 10, 2643-2655 (October 1995)). The major dose-limiting side effect of paclitaxel is neutropenia. Other side effects include dose dependent mucositis and peripheral neuropathy, cardiac rhythm abnormalities, arthralgias/myalgias, hypersensitivity reactions, alopecia, nausea and vomiting. The major dose limiting side effect of docetaxel is neutropenia. Other side effects include paresthesias, hypersensitivity reactions, alopecia, skin reactions, fluid retention, nausea, vomiting and diarrhea. A discussion of the side effects experienced with paclitaxel and docetaxel (TAXOTERE®), may be found in the *Physician's Desk Reference, supra*. These side effects may be defined and graded using the common toxicity criteria of the U.S. National Cancer Institute or COSTART classification. The patient's AAG level may be used to predict the possibility of a variety of side effects, in particular, grade 4 neutropenia, infection and grade 3 diarrhea.

The present invention provides methods for assessing the effect of treatment as it relates to side effects for a patient who has cancer and who is being treated with a taxoid. The method involves observing the patient's AAG level, classifying the AAG level as high, intermediate or low compared to predetermined AAG levels derived from a population of patients having the same type of cancer and being treated with the same taxoid at a common dosage, and based on this comparison assessing the side effects that may be experienced by the patient.

The present invention also provides a method for assessing whether a patient who has cancer and who is to be treated with a taxoid will experience side effects. The method involves observing the patient's AAG level prior to

tr atment and comparing this level to a predetermined AAG level d rived from a population of patients having the same type of cancer and being treated with the same taxoid that is to be used in treating the patient, and, based on this comparison recommending a dosage of the taxoid that will reduce or eliminate side effects that may be experienced by the patient, while providing an improvement or cure in the patient's condition.

The odds of experiencing side effects resulting from taxoid treatment can be predicted based on AAG levels. The relationship between AAG levels and the occurrence of side effects is believed to relate to the AAG-taxoid binding interaction. In accordance with the present invention, it has been found that patients with high AAG levels are less likely to experience adverse side effects than patients with low AAG levels.

If a quantitative characterization of the relationship between AAG and side effects is desired for a particular type of cancer, one of skill in the art can readily obtain blood samples from a population of patients having a given type of cancer and follow the guidance provided in the Examples below to further define the relationship between AAG levels and side effect(s) due to treatment with a given taxoid.

Determination of Dosage Levels

Based on the ability to predict response to treatment, survival, and side effects, the present invention may be used to adjust the dosage of a taxoid being administered to a patient. Accordingly, the present invention provides methods for determining the dosage of a taxoid to administer to a patient being treated for a cancer. These methods involve observing the patient's level of AAG and evaluating the AAG level to determine the dosage of the taxoid to administer to the patient by comparing the patient's AAG level to a predetermined AAG level in a population of patients who have the same type of cancer and who are being treated with the same taxoid at a common dosage. Based on this information, a

recommendation can be made on the dosage of taxoid to give to the patient. With regard to response rate, a patient who has a high AAG level and who is predicted to have a decreased chance of responding to treatment may have their taxoid dosage increased. Similarly, a patient who has a high AAG level and who is predicted to have a reduced length of survival may have their taxoid dosage increased. Given that patients with high levels of AAG are also less likely to experience side effects, it may be possible to increase the AAG dosage for these patients without a corresponding increase in the possibility of side effects.

Given the correlation between AAG levels and side effects, one method for reducing the possibility of side effects is to determine the patient's AAG level and to adjust the taxoid dose so as to reduce the possibility of side effects.

Accordingly, the present invention also provides methods for reducing the chance of a cancer patient's experiencing side effects from a taxoid by observing the patient's level of AAG prior to treatment and classifying the patient's level of AAG as high or low. If the patient has a low AAG level, the dose of a taxoid to be administered to the patient may be reduced so as to reduce or eliminate any possible side effects.

The dosage levels for taxoids are specific to the particular taxoid being used and the cancer being treated. Dosage recommendations for the clinically available taxoids are provided with the products and may also be found in the Physicians' Desk Reference and in the scientific literature. Dosage recommendations for the taxoid docetaxel range from about 55 mg/m^2 to about 125 mg/m^2. These dosages are usually administered intravenously over 1 hour every three weeks. Dosage recommendations for the taxoid paclitaxel range from about 135 mg/m^2 to about 175 mg/m^2. These dosages are usually administered intravenously over 3 hours every three weeks.

Guidance for adjusting taxoid dosage based on actual side effects may be found in the *Physician's Desk Reference*, 52nd ed., (1998) (for TAXOL® (paclitaxel) see p762-766 and for TAXOTERE® (docetaxel) see p2385-2389). These recommended adjustments based on actual side effects may be used as a guide to adjusting dosage based on predicted side effects.

With regard to paclitaxel, patients who have low AAG levels and would be predicted to experience neutropenia or other side effects such as infection or grade 3 diarrhea during paclitaxel therapy may have their paclitaxel dosages reduced by about 5 to about 35%, preferably by about 10 to about 30%, even more preferably from about 15 to about 27%.

With regard to docetaxel (Taxotere®), patients who would be predicted to experience neutropenia, including febrile neutropenia or other side effects may have their docetaxel dosage reduced by about 5 to about 35%, preferably by about 10 to about 30%, even more preferably about 15 to about 27%. If the side effects actually occur, the dosage may be further decreased.

In general, the taxoid dosage may be adjusted upwardly, or downwardly, based on actual side effects and response to treatment.

Description of an Embodiment of the Invention

The methods of the present invention are illustrated in the Examples below which describe a study involving NSCLC cancer patients who were treated with the taxoid docetaxel (Taxotere®). The study involved a determination of the relationship between AAG levels and response to treatment, survival, and side effects.

The study included 180 NSCLC patients who were enrolled in six Phase II studies of 100 mg/m^2 of docetaxel.

The AAG levels of the patients were determined and classified into high (≥1.85 g/L (75 percentile and above)),

intermediate (1.12 to 1.84 g/L (26 percentile to 74 p rcentile), and low (≤1.11 g/L (25 percentile and below) levels.

The general relationship between AAG levels and response, survival, and side effects that were observed in NSCLC patients treated with the taxoid docetaxel can be summarized as follows: (a) patients with low AAG levels have a greater response rate to treatment with a taxoid than patients with high AAG levels; (b) patients with low AAG levels being treated with a taxoid survive longer than patients with high AAG levels; and (c) patients with low AAG levels will be more likely to experience adverse side effects from taxoid treatment relative to patients with high AAG levels.

As presented in the Examples below, the study shows that a patient having a low AAG level (≤1.11 g/L) had a response rate of 41.3% compared to a 15.9% response rate for patients with high (≥1.85 g/L) AAG levels. Accordingly, a patient's AAG level can be determined using the methods described above, preferably the Bienvenu et al. method, and the patient's observed AAG level may be compared to the population's predetermined AAG levels and the patient's AAG level classified into a low, intermediate, or high category. If the patient has a low AAG level, it can be predicted that the patient will have an increased chance of response to treatment. Similarly, if the patient's AAG level falls into the high AAG level category, it can be predicted that the patient will have a reduced chance of responding to treatment with a taxoid. Based on these predictions, treatment options may be considered, including, for example, maintaining the treatment at the current taxoid dosage, adjusting the taxoid dosage and/or expanding the treatment to include additional chemotherapeutic, surgical, or radiological treatment.

With regard to survival, patients having a low AAG level (≤1.11 g/L) had a median survival of 15.6 months. Patients having an intermediate AAG level (1.12 to 1.84 g/L) of AAG

had a median survival of 9.2 months and patients with a high level (≥1.85 g/L) of AAG had a median survival of 5.5 months. Accordingly, a patient's AAG level can be determined using the methods described above, preferably the Bienvenu et al. method and the patient's observed AAG level may be classified as having a low, intermediate, or high level of AAG compared to the population's predetermined AAG levels. Based on the patient's AAG level, the patient may be predicted to have a period of survival measured from the initiation of taxoid treatment to be of long, intermediate, or short duration. For example, a patient having a low AAG level would be expected to have a longer survival than a patient with intermediate or high levels or AAG. For patients with intermediate or high AAG levels, treatment options may be considered, including, for example, maintaining the treatment at the current taxoid dosage, adjusting the taxoid dosage and/or expanding the treatment to include additional chemotherapeutic, surgical, or radiological treatment.

With regard to side effects, as the AAG level varied from low (≤1.11 g/L) to high (≥1.85 g/L), there was approximately a 50% reduction in the odds of experiencing an adverse side effect (febrile neutropenia or infection or grade 3 diarrhea). Accordingly, a patient's AAG level can be determined using any of the methods described above, preferably the Bienvenu et al. method, and the patient's observed AAG level can then be classified as low, intermediate, or high AAG level compared to the population's predetermined AAG levels. If the patient's AAG level is in the low range, it can be predicted that the patient will have an elevated chance of experiencing side effects. If the patient is predicted to have an elevated chance of experiencing side effects, consideration can be given to lowering the patient's dose of the taxoid so as to reduce the chances of undesirable side effects. Reduction of the dosage of the taxoid must be balanced against reduction in the efficacy of treatment.

Examples

The following examples are representative of the practice of the invention.

Example 1

This example is illustrative of the present invention. It provides information stemming from a study of cancer patients who were treated with a taxoid (docetaxel) and concerning the relationship between AAG levels and a variety of physiological effects, including, for example, side effects. This study in its entirety is reported in applicant's U.S. provisional patent Application No. 60/114,520, filed December 30, 1998, which is incorporated herein by reference. The results of this study were published in Bruno et al., *Journal of Clinical Oncology*, Vol. 16, No. 1, p. 187-196 (1998).

The Patient Pool

Data were prospectively collected from patients entered in twenty-four Phase II open, non-randomized studies conducted from May 1992 to March 1994 to assess docetaxel clinical efficacy in a variety of tumor types including breast cancer, non small cell lung cancer, ovarian cancer, head and neck cancer, melanoma, renal cancer, colorectal cancer, gastric cancer, small cell lung cancer, and soft-tissue sarcoma. The studies were conducted in over 50 centers in Europe and three centers in the United States.

Criteria for eligibility included histology, at least one bidimensionally measurable lesion, adequate bone marrow reserve (absolute neutrophil count > 2,000/μL), adequate renal function (normal creatinine level) and liver function (total bilirubin level < 1.25 x ULN, SGOT (ALT) $\leq$2 x ULN or $\leq$3 x ULN in case of proven liver metastases). According to the tumor type, patients could have received various extents of prior treatment. The starting dose of docetaxel was either 75 mg/m^2 or 100 mg/m^2 given as a 1-hr infusion every 3 weeks. Dose reduction (25 %) or delay of subsequent courses were permitted, based on the degree of toxicity observed.

Most of the patients who registered (721/936, 77%) were sampled and among them, 81 were not considered valuable for the study for the following reasons: not sampled at the first course (n=12, 1.7%); lack of documentation of samples (n=32, 4.4%); samples lost during transfer from the clinical sites to the analytical laboratory (n=18, 2.5%); or during assay procedure (n=19, 2.6%). Overall, 640 patients (89% of patients sampled, 68% of patients treated) were evaluable at first course.

Measurement of AAG Levels

AAG levels were determined by a variety of methods, primarily by the Bienvenu et al. laser nephelometry method. (See Bienvenu et al. Clinical Chemistry, 27(5), 721-726 (1981) and Example 3.)

Sampling Strategy

The aim of the sampling strategy in connection with taking blood samples from the patients was to define the full pharmacokinetic profile over the population, the so called "full screen" approach (Sheiner LB, Benet L Z: Premarketing observational studies of population pharmacokinetics of new drugs. Clin Pharmacol Ther 38: 481-487, 1985), by drawing a few samples per patient and varying (randomizing) the sampling times among patients (Hashimoto Y, Sheiner LB: Designs for population pharmacodynamics : Value of pharmacokinetic data and population analysis. J Pharmacokinet Biopharm 19: 333-353, 1991).

Recognizing the goal of individual estimates, the sampling strategy design was based on optimal individual sampling times computed using preliminary population PK parameter estimates obtained from Phase I data (Launay-Iliadis MC, Bruno R, Cosson V, et al: Population pharmacokinetics of docetaxel during Phase I studies using nonlinear mixed-effect modeling and nonparametric maximum-likelihood estimation. Cancer Chemother Pharmacol 37: 47-54, 1995). The sampling times were D-optimal (D'Arg nio DZ: Optimal sampling times for pharmacokinetic experiments. J

Pharmacokinet Biopharm 9:739-756, 1981) and were computed using the APIS package, version 3.03a (Iliadis A, Brown AC, Huggins ML: APIS : A software for model identification, simulation and dosage regimen calculations in clinical pharmacokinetics. Comput Methods Programs Biomed 38: 227-239, 1992). Recognizing the goal of population estimates, separate sampling schedules, each consisting of early, mid and late time samples, were used to assure that the population PK samples were well spread across the available sampling time range.

There were 6 D-optimal sampling times (OST) for a three-compartment PK model (involving 6 parameters). OSTs were computed over a 0-24 hours observation interval. The estimated times (h:min) were: 0:30 (mid-infusion) or 1:00 (end of infusion), 1:15, 1:45, 3:45, 8:20 and 24:00.

The blood-sampling strategy consisted of four different sampling schedules (Table 1 below) which were assigned randomly to patients at study entry. Each schedule consists of 3 sampling times ranging between mid-infusion and 6 hours (5 hours post infusion). The first sample was always taken during the infusion, either mid infusion or just (5 minutes) before the end of the 1 hour-infusion. The two other samples were drawn within 5 hours after the end of infusion. Six hours was the maximum observation time in order to comply with outpatient status. However, when possible (e.g. for inpatients), one point could be replaced by a late sample drawn any time between 12 and 24 hours. A predrug sample (optional) was also requested to check the absence of analytical interference in patient plasma.

TABLE 1

SAMPLING STRATEGY IMPLEMENTED IN PHASE II STUDIES

Sampling Times			
Sampling Schedule No.	1	2*	3
	During Infusion	After Infusion	
		Minutes	Hours
1	5 minutes before end	10	2
2	30 minutes after start	20	3
3	5 minutes before end	30	4
4	30 minutes after start	60	5

* When possible, this sample will be replaced by a blood sample obtained at a later time, i.e., any time between 12 and 24 hours post infusion.

A pharmacokinetic case report form (PK CRF) was designed to document actual sampling times as well as actual time of beginning and end of infusion. In some patients experiencing infusion-related hypersensitivity reactions, administration was interrupted and then resumed shortly after (e.g. 30 minutes). Actual times of starting and stopping the 2nd infusion were also documented on the PK CRF. Docetaxel was assayed in plasma samples using high performance liquid chromatography and UV detection after solid-phase extraction (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992) in 2 different cross-validated centers.

Pharmacokinetic Data Analysis

The collected data permitted elaboration and validation of a population pharmacokinetic model relating docetaxel clearance to patho-physiologic factors. This analysis has recently been reported (Bruno R., Vivier N., Vergniol J.C., et al: A population pharmacokinetic model for docetaxel (Taxotere®) : Model building and validation. J Pharmacokinet Biopharm 24:153-172, 1996). Population parameters from this analysis were used as prior information to estimate each

individual's pharmacokinetic parameters from his plasma concentrations using Bayesian estimation as implemented in the NONMEM computer program (version IV, level 2.0) (Beal S L, Boeckman AJ, Sheiner LB. NONMEM. User's Guide Part I to VI. University of California at San Francisco, San Francisco, 1988 - 1992).

The PK model was a three-compartment structural model with first-order elimination. The basic parameters were elimination clearance (CL, L/h), volume of distribution of the central compartment and intercompartmental rate constants. The inter-patient variability of PK parameters was modeled as (e.g. for CL):

$$CL_j = \hat{CL}_j \exp(\eta_{jCL})$$

where η_{jCL} denotes the (proportional) difference between the true parameter (CL_j) of individual j and the typical value in the population $\hat{CL}_j$ according to covariable values affecting $\hat{CL}$ for the j^{th} individual. Residual variability was modeled as proportional, consistent with the constant coefficient of variation of the assay measurement error (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992).

Individual plasma clearance (CL_j), area under the plasma concentration-time curve (AUC_j), peak plasma level, and time that plasma levels were greater than given threshold levels were used as measures of drug exposure.
CL_j was directly estimated by the Bayesian CL after fitting. Based on the estimate of CL_j, the following clearance factor (CLf) was generated:

$$CLf_j = (\text{mean CL}) / CL_j$$

Note that CLf_j is inversely proportional to CL_j : it takes values less than 1 for patients with clearance greater than the mean, and values greater than 1 for patients with clearance less than the mean (e.g. 2.0 for a 50 % decrease in clearance). Use of this derived parameter facilitates the

interpretation of pharmacokinetic/pharmacodynamic (PK/PD) models in term of clearance changes, as discussed below. AUC_j is computed as :

AUC_j ($\mu g \cdot h/mL$) = $Dose_j$ (mg) / CL_j (L/h)

Peak plasma level was taken to be the model predicted concentration at the end of infusion. Duration of exposure to plasma levels greater than 0.16 μg/mL (0.20 μM) ($t_{0.20}$), 0.080 μg/mL (0.10 μM) ($t_{0.10}$) and 0.040 μg/mL (0.05 μM) ($t_{0.05}$) was computed from estimated parameters using the implicit equation solver of EXCEL spread sheet, version 5 (Microsoft Corporation).

PK/PD analysis was conducted using as independent variables individual estimates, CLf_j, other exposure parameters (see above) and several other covariables related to the patient's patho-physiological status (demographics, disease spread) and extent of prior treatment. Docetaxel dose (mg/m^2), either given at first course or cumulative, was also considered as an independent variable measuring drug exposure.

Objective response rate, time to first response, and time to progression were selected as the efficacy endpoints (dependent variables). Only data from patients with breast cancer and non-small cell lung cancer (NSCLC) were analyzed. Assessment of tumor response was made every six weeks according to WHO criteria. Objective responses (complete responses (CR) and partial responses(PR)) were confirmed after a minimum of 4 weeks and were reviewed by an independent panel. Time to first response was calculated from the first docetaxel infusion up to the date of the first objective response either CR or PR whichever occurred earlier. Time to progression was calculated from the first docetaxel infusion up to the date of progression.

For safety, the following endpoints were considered among all tumor types:

- Neutropenia (NCI Grade) at first course.
- Febrile neutropenia at first course. Febrile neutropenia was defined as fever > 38°C (NCI grade ≥ II) with a

concomitant NCI grade 4 neutropenia (neutrophil count < 500/ μL) requiring antibiotics and/or hospitalization.
- Time to onset of fluid retention calculated from the first docetaxel infusion up to the date of the first sign and/or symptom of fluid retention (peripheral edema, pleural or pericardial effusions, ascites or weight gain).

Logistic regression was used to relate categorical endpoints, such as response rate and neutropenia grade, to the independent variables, while Cox regression was used for time to first response, time to progression and time to onset of fluid retention. Dose was the only time-dependent covariate in the Cox model. Model development involved stepwise inclusion and deletion of covariates. Significance levels for variable entry or removal at each step was $P < 0.10$; however, a final elimination pass, using $P < 0.05$ was used to determine the covariates kept in the final model. The median time to onset of fluid retention was estimated using the Kaplan-Meier method. Analyses were carried out using the SAS software (SAS version 6.11; SAS Institute Inc., Cary, NC).

Discussion of Results

Typical individual PK profiles are shown in Fig. 1 illustrating two of the four sampling schedules. The full population PK profile achieved by varying the sampling scheme across patients is illustrated in Figure 2 (data from a subset of 254 patients). This profile comprises 716 data points, that is, a mean of 2.8 per patient (range 1 to 5). Overall, a fair number of late samples was obtained (67 samples over 50 patients).

Patient characteristics at baseline

Patient characteristics are summarized in Table 2. Median age was 56 years, 42% were males and 58% females, 231 patients (36%) had breast cancer and 189 (30%) had NSCLC. Thirty-two percent of the patients were asymptomatic (WHO performance status of 0), whereas performance status of 1 and 2 were reported in 54% and 14% of the patients respectively. Thirty-three percent of patients had ≥ 3 organs involved, 82

% had visceral metastases, 35 % had liver metastases and 45% had previously been treated with chemotherapy. Most of the patients (95%) received 100 mg/m² as initial dose. Initially no premedication was used. Various premedication regimens (anti-H1 ± anti-H2 and/or corticosteroids either short term (≤ 2 days) or long term (≥ 3 days)) were subsequently given in some studies to prevent hypersensitivity reactions and fluid retention occurring during treatment. Few patients (n=25, 3.9%) received the five-day dexamethasone, presently recommended, premedication (8 mg orally twice daily starting the day before docetaxel administration).

TABLE 2

PATIENT CHARACTERISTICS AND DOCETAXEL EXPOSURE (N=640)

	COUNT		MEDIAN	5% TO 95% PERCENTILE
	NO.	%		
Age, years			56	38-71
Sex Male Female	 270 370	 42 58		
WHO performance status 0 1 2	 202 342 90	 32 54 14		
Total protein (g/L)			71	59-81
Albumin (g/L)			41	31-48
AAG (g/L)			1.34	0.76-2.59
Elevated liver enzymes	26	4.1		
Tumor type Breast NSCLC Other	 231 189 220	 36 30 34		
Disease spread No. of disease sites ≥3 Visceral involvement (yes) Liver metastasis (yes)	 214 522 221	 33 82 35		
Prior treatments Chemotherapy (yes) No. of prior regimens (≥2)	 289 110	 45 17		
Taxotere treatment/exposure Initial dose (mg/m²) 75 100	 31 609	 5 95		
CL (L/h)			36.3	17.5-59.3
CLf			1.02	0.622-2.11

	COUNT		MEDIAN	5% TO 95% PERCENTILE
	NO.	%		
AUC (μg-h/mL)			4.81	2.93-9.52
Peak (μg/mL)			3.26	1.93-5.76
$t_{0.20}$ (hours)			2.41	1.52-6.16 (0.858†)
$t_{0.10}$ (hours)			3.65	2.24-16.7 (0.856†)
$t_{0.05}$ (hours)			9.60	3.38-30.7 (0.838†)
Premedication				
None	252	39		
Recommended (5 days dexamethasone)	25	5		
Other	363	57		

* Patients with concomitant elevations of transaminases (>1.5 x ULN) and alkaline phosphatase (>2.5 x ULN).

† Correlation coefficient with AUC.

Individual PK parameter estimates

Individual estimates of PK and exposure parameters are given in Table 2. The continuous lines in Fig. 1 denote fits of patient data obtained using Bayesian estimation. In this large patient population, median clearance was 36.3 L/h which is a value very close to the value of 35.6 L/h previously estimated from Phase I data (Launay-Iliadis et al. Cancer Chemother Pharmacol 37:47-54 (1995)) and varied from 17.5 L/h to 59.3 L/h (5% to 95% percentile range). Representative exposure parameters were AUC: 4.81 μg·mL/h and peak: 3.26 μg/mL. Duration of exposure greater than threshold levels varied from 2.41 hours (0.20 μmol/L) to 9.60 hours (0.05 μmol/L). All of the measures of duration of exposure were strongly correlated with AUC $r \geq 0.838$, Table 2).

Pharmacokinetics/Pharmacodynamics - Efficacy

No significant relationship was found between any estimate of docetaxel exposure and either objective response rate, time to first response or time to progression in breast cancer (201 evaluable patients, response rate: 56%). The number of disease sites was a significant predictor of response for all endpoints ($p<0.05$), baseline alpha-1-acid glycoprotein level (AAG) and number of prior chemotherapy

regimens were additional predictors ($p<0.005$) of time to progression.

Regarding NSCLC (151 evaluable patients, response rate : 29%), docetaxel AUC at first cycle was a significant predictor ($p=0.0232$) of time to progression after adjusting for other covariates (see Table 3). AUC was the only measure of docetaxel exposure to reach statistical significance. The median time to progression was 99 days (95% confidence interval: 84 - 121 days). According to this model, the risk of progression is decreased by 11 % per unit AUC and by 43 % for 5 AUC units (e.g. from the median to about the 95 percentile in this population). In addition, duration of exposure over 0.10 µmol/L was the only measure of exposure to reach borderline statistical significance ($p\sim0.10$) in predicting either response rate or time to first response. Of note, baseline AAG was a significant predictor of response for all endpoints ($P<0.005$).

TABLE 3

NSCLC: COX REGRESSION MODEL FOR TTP (N=151)

PREDICTOR	P	RISK RATIO	95% CI
Cumulative dose*	.0002	0.997	0.995-0.998
No. of disease sites	.0011	1.293	1.109-1.507
AAG	.0022	1.757	1.225-2.518
Performance status	.0177	1.483	1.071-2.055
AUC	.0232	0.891	0.807-0.984

Note: Progression occurred in 84% of patients (127 of 151).
Abbreviation: CI, confidence interval.

* Time-dependent covariate.

Neutropenia

Neutropenia was analyzed at first cours in 582 patients. Most of the patients (375/582, 64%) experienced grade 4 neutropenia. Several strong predictors of grade 4

neutropenia were identified including the various measures of docetaxel exposure with Clf, AUC and $t_{0.20}$ having the strongest effects (P<0.0001). After adjustment for the other covariates in the model, dose no longer had a significant effect. Clearance factor, CLf was retained in the final model (Table 4 below) since it greatly facilitates the interpretation of the model in terms of clearance change. The incidence of neutropenia grade 4 was related to the baseline neutrophil count (P=0.0002) and the number of previous regimens (P=0.0002) as expected. Baseline AAG level and first course exposure were the most significant predictors (P<0.0001). The higher the AAG level at baseline, the lower the odds of experiencing grade 4 neutropenia during the first course of treatment. According to the logistic regression model, a 1-g/l increase of baseline AAG (for example, from the median to about the 95 percentile in this population) results in a 83% decrease in the odds of experiencing grade 4 neutropenia. The effect of drug exposure change is the opposite with a 430% (4.3 fold) increase of the odds of grade 4 for a 1 unit increase in CLf. A 1 unit increase in CLf corresponds to a 50% decrease of clearance which is also a change from the median to the 95th percentile in this population).

TABLE 4

LOGISTIC REGRESSION MODEL FOR GRADE 4 NEUTROPENIA (N=582)

PREDICTOR	P	ODDS RATIO	95% CI
AAG	<.0001	0.17	0.10-0.29
Clf	<.0001	4.26	2.46-7.39
Baseline count	.0002	0.84	0.77-0.92
No. of previous regimens	.0002	1.72	1.30-2.29

Note: Incidence, 64% (375 of 582 patients)

Febrile neutropenia was observed in 26 of the 582 patients (4.5%) at first cycle. The model for this endpoint

was similar to that for neutropenia grade 4 with exposure (CLf) and AAG being the only significant predictors (Table 5 below). In this model, change of exposure due to a 50% decrement in clearance would result in a 300% (3.0 fold) increase in the odds of febrile neutropenia. The model-predicted probability of febrile neutropenia as a function of Clf (AAG fixed at the median) is illustrated in Figure 3.

TABLE 5

LOGISTIC REGRESSION MODEL FOR FEBRILE NEUTROPENIA (N=582)

PREDICTOR	P	ODDS RATIO	95% CI
Clf	.0012	3.03	1.55-5.93
AAG	.0056	0.28	0.12-0.69

Note: Incidence, 4.7% (26 of 582 patients).

Fluid retention

Fluid retention occurred in 53% of 631 evaluable patients. The median time to onset was 85 days (95% confidence interval: 81 to 92 days). Patients with breast and ovary carcinoma had disease related baseline symptoms resulting in a higher baseline risk than patients with other tumor types. The analysis was stratified, therefore, by tumor type with breast and ovary combined and other tumor types combined. Fluid retention incidence was 73% (172 of 236) in patients with breast or ovary tumors and 41% (163 of 395) in patients with other tumor types. Of note, few patients (n=25, 4%) received the presently recommended 5-day dexamethasone premedication in this population since this premedication was only recommended after the majority of these patients had been treated.

Owing to the cumulative nature of docetaxel induced fluid retention, dose was treated as a time-dependent covariate in the analysis. Cumulative dose was the most important predictor in the final Cox regression model (Tabl 6 below). However, several other baseline covariates had independent predictive power including AAG and total protein

levels. Drug exposure at first course was also highly significant in predicting the time to onset of fluid retention, after adjustment for the effect of cumulative dose. The duration parameter, $t_{0.20}$ was the most significant (P=0.0029) measure of exposure for this regression.

TABLE 6

COX REGRESSION MODEL FOR TIME TO ONSET OF FLUID RETENTION

(N=631)

PREDICTOR	P	RISK RATIO	95% CI
Cumulative dose*	<.0001	1.005	1.003-1.007
$t_{0.20}$	.0029	1.087	1.029-1.148
Total protein	.021	0.980	0.964-0.997
AAG	.014	0.746	0.591-0.942

Note: Incidence, 53% (335 of 631 patients).

Stratification: breast/ovary-236 patients/incidence, 73%; other-395 patients/incidence, 41%.

*Time-dependent covariate.

According to the model, the risk of experiencing fluid retention at any time is increased by 64% for each additional cycle at 100 mg/m². An increase of $t_{0.20}$ by 4 hours i.e. roughly from the median (2.41 hours) to the 95th percentile (6.16 hours) at first course increases the risk by 40% beyond the effect of cumulative dose.

Baseline AAG level was a significant predictor of all the PD endpoints investigated in this study.

Example 2

This example is illustrative of the present invention. It provides information stemming from a study of alpha-1-acid glycoprotein as an independent predictor of response and survival in patients with non-small cell lung cancer treated with docetaxel.

The Patient Pool

The data for this study was prospectively collected from unresectable and metastatic NSCLC patients entered into six Phase II open label, non-randomized studies of docetaxel (Burris H, Eckardt J, Fields S, et al: Phase II trials of Taxotere in patients with non small cell lung cancer. Proc Am Soc Clin Oncol 12: 335, 1993 (abstr 1116); Cerny T, Kaplan S, Pavlidis N, et al: Docetaxel (Taxotere) is active in non-small-cell lung cancer: A phase II trial of the EORTC Early Clinical Trials Group. Br J Cancer 70: 384-387, 1994; Fossella FV, Lee JS, Murphy WK et al: Phase II trial of docetaxel for recurrent or metastatic non-small cell lung cancer. J Clin Oncol 12: 1238-1244, 1994; Francis PA, Rigas JR, Kris MG et al: Phase II trial of docetaxel in patients with Stage III and IV non-small cell lung cancer. J Clin Oncol 12: 1232-1237, 1994; Fossella FV, Lee JS, Shin DM, et al: Phase II study of docetaxel for advanced or metastatic platinum-refractory non-small-cell lung cancer. J Clin Oncol 13: 645-651, 1995; Miller VA, Rigas JR, Francis PA, et al: Phase II trial of a 75 mg/m^2 dose of docetaxel with prednisone premedication for patients with advanced non-small cell lung cancer. Cancer 75: 968-972, 1995). Detailed information and clinical trial results for these studies have been previously reported.

The criteria for eligibility included confirmation of non-small cell lung cancer, one or more bidimensionally measurable lesion, adequate bone marrow (absolute neutrophil count > 2,000/mL), renal (normal creatinine) and hepatic function (total bilirubin < 1.25 x upper limit of normal (ULN), alanine aminotransaminase (ALT) ≤ 2 x ULN). According to the study design, patients may have received prior treatment. The initial docetaxel dose for most patients was 100 mg/m^2 given as a 1-hr infusion every 3 weeks. Dose reduction of twenty-five percent or delay of subsequent courses of therapy was permitted, based on the grade of toxicity observed. These studies were part of the 22 Phase II studies reported in a previous PK/PD analysis of docetaxel (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics

Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998).

Measurement of AAG Levels

AAG levels were determined by a variety of methods, primarily by the Bienvenu et al. laser nephelometry method. (See Bienvenu et al. Clinical Chemistry, 27(5), 721-726 (1981) and Example 3.)

Pharmacokinetic Data

Pharmacokinetic assessment was performed at the first cycle of treatment. The design of the sampling strategy was presented in Example 1 and in detail in Bruno *et al* (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics /Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998). Briefly, the sampling strategy consisted of four different sampling schedules of 3 sampling times which were randomly assigned to patients upon study entry. Docetaxel was assayed in plasma samples using high performance liquid chromatography and UV detection after solid-phase extraction (Vergniol JC, Bruno R, Montay G et al: Determination of Taxotere in human plasma by a semi-automated high-performance liquid chromatographic method. J Chromatog 582: 273-278, 1992).

From the population pharmacokinetic parameters (Bruno R, Vivier N, Vergniol JC et al: A population pharmacokinetic model for docetaxel (Taxotere®) : Model building and validation. J Pharmacokinet Biopharm 24:153-172, 1996), Bayesian methods were used to estimate each individual's pharmacokinetic parameters from the patient's plasma concentrations (Baille P, Bruno R, Schellens JHM et al: Optimal sampling strategies for Bayesian estimation of docetaxel (Taxotere®) clearance. Clin Cancer Res 3:1535-1538, 1997). The NONMEM computer program was employed for these studies (version IV, level 2.0) (Beal SL, Boeckman AJ, Sheiner LB. NONMEM. User's Guide Part I to VI. University of

California at San Francisco, San Francisco, 1988 - 1992). The PK model used a three-compartment structural model with first-order elimination and the PK parameters considered for this analysis are CL, and AUC.

Clinical Endpoints

The following clinical endpoints were considered for this analysis.

Safety : Febrile neutropenia, infections, grade 3/4 stomatitis, grade 3/4 diarrhea and severe asthenia, reported during the first course of therapy were considered as safety endpoints. These parameters were selected as they typically require dose reduction or treatment delay. Stomatitis and diarrhea were defined and graded using the Common Toxicity Criteria of U.S. National Cancer Institute whereas COSTART classification was used for asthenia. Febrile neutropenia was defined as body temperature > 38°C with concomitant NCI grade 4 neutropenia (neutrophil count < 500/mL) requiring antibiotics and/or hospitalization.

Due to the small number of patients and low incidence of severe adverse events, these safety endpoints were pooled for analysis.

Response rate : The patients were considered to be a responder when they experienced either a partial response (PR) or a complete response (CR) using standard criteria. Patients with minor responses (< 50 % reduction in tumor size), evaluable disease, stable disease and patients with disease progression were considered as non responders. Responses had to be confirmed after a minimum of 4 weeks and were reviewed by an independent panel.

Survival : Survival was calculated from the date of the first infusion to the date of death, last contact for patients lost of follow-up, or a cut-off date for patients alive at the time of closure of the data set.

Data Analysis

Three categories of independent variables thought to affect survival in NSCLC were considered for this analysis. Firstly, docetaxel exposure as assessed by the cumulative dose, or CL and AUC at first course Secondly, the patient characteristics including age, gender, performance status, alpha-1-acid glycoprotein, lactate dehydrogenase, baseline neutrophil count, time from initial diagnosis of NSCLC, number of disease sites, visceral cancer involvement, hepatic metastasis and bone metastasis. Thirdly, the extent of prior treatment reported as prior chemotherapy, number of prior chemotherapy regimens, prior cisplatin, and prior radiotherapy.

A logistic regression was used to relate binary endpoints, such as the incidence of severe adverse events and response rate, to the independent variables, while a Cox regression was used for the survival analysis. Cumulative docetaxel dose was the only time-dependent covariate used in the Cox model. Univariate and multivariate analyses were conducted. The multivariate model involved a stepwise selection of covariates starting from the null model. Significance levels for variable entry or removal at each step in the development of the multivariate model were $p<0.10$ and $p<0.05$, respectively. The median survival was estimated using the Kaplan-Meier method. Analyses were carried out using the SAS software (SAS version 6.12; SAS Institute Inc., Cary, NC).

Discussion of Results

Patient characteristics at baseline

Overall, 189 patients of the 269 NSCLC patients entered in the six Phase II studies of doc taxel (70 %) had pharmacokinetic data available for analysis. Nine patients received 75 mg/m^2 of docetaxel as their initial dose, and all other patients (n=180) received 100 mg/m^2. This analysis was restricted to the patients treated with 100 mg/m^2 of

docetaxel. Among these patients, 143 were evaluable for response, however, the analysis was conducted on the intent-to-treat population of 189 patients. Some models were reassessed on the evaluable patient population as a sensitivity analysis to examine their affects on the predictor outcomes. The patient characteristics are summarized in **Table 7**. Median age for this population of NSCLC patients was 61 years, two thirds were male, 82% of the patients had a WHO performance status of 0 to 1. Most of the patients were chemotherapy naive (71%), and had metastatic disease (77%).

Table 7. Patient characteristics and docetaxel exposure (n = 180)

percentile		Number	%	median	5%-95%
Age (years)				61	43-72
Sex	Male			118 (66)	
	Female			62 (34)	
WHO performance status		0		35 (19)	
		1		113 (63)	
		2		32 (18)	
α1-acid glycoprotein (g/l)				1.42	0.84-2.71
Time from diagnosis (month)				4.7	0.6-39
	>12 month			48 (27)	
<u>Extent of disease</u>					
Number of disease sites		1		48 (27)	
		2		70 (39)	
		3		42 (23)	
		≥4		20 (11)	
Liver metastasis				34 (19)	
<u>Prior treatments</u>					
Chemotherapy				52 (29)	
Number or prior regimen		0		128 (71)	
		1		34 (19)	
		≥2		18 (10)	

percentile	Number % median	5%-95%
Prior platinum	43 (24)	
Radiotherapy	70 (39)	
Docetaxel exposure		
CL (L/h)	35.7	17.8-58.8
AUC (mg.h/mL)	4.98	3.24-9.76

Individual PK parameter estimates

Individual estimates of PK and exposure parameters are given in **Table 7.** In this NSCLC patient population, the median clearance was 35.7 L/h varying from 17.8 L/h to 58.8 L/h (5% to 95% percentile range). This clearance distribution was very similar to that of the larger population of patients with various tumor types with a median of 36.3 L/h (Bruno R, Hille D, Riva A, et al: Population Pharmacokinetics /Pharmacodynamics (PK/PD) of Docetaxel in Phase II studies in patients with cancer. J Clin Oncol 16:187-196, 1998); The observed median AUC was 4.98 mg·mL/h with a 5% to 95% percentile range 3.24 mg·mL/h to 9.76 mg·mL/h.

Severe adverse events

Twenty-five patients (13.9 %) experienced at least one severe adverse event during the (TOX) first cycle of therapy (**Table 8**). Docetaxel exposure as measured by the AUC was the only significant predictor of these adverse events ($p<0.0001$). A high AUC was associated with increased probability of experiencing any of the severe toxicities. Age of the patients had a borderline significant effect ($p = 0.056$), with older patients showing a trend towards a higher probability of experience a severe adverse event.

Table 8. Incidence of adverse events at cycle 1

	number	%
Febrile Neutropenia	7	3.9
Infection	8	4.4
Stomatitis (Grade 3, 4)	3	1.7
Diarrhea (Grade 3, 4)	10	5.6
Asthenia (severe)	2	1.1
Endpoint		
TOX*	25	13.9
TOX1**	23	12.8

*patients experienced at least one event

**patients experienced febrile neutropenia or infection or grade 3 diarrhea

Subsets of associated toxicities were also analyzed for their correlative significance. In all subsets, AUC was the only significant predictor of these severe adverse events. In one subset that included febrile neutropenia or infection or diarrhea (TOX1), with 23 adverse events, (12.8%), AAG reached borderline significance (p=0.0505) in addition to AUC.

The odds ratio for the logistic regression models calculated for the relevant covariate changes from the 25th to the 75th percentiles are given in **Table 9**. According to the logistic model, the odds of experiencing a severe adverse reaction was approximately 2 fold greater for a change in AUC from 4.2 to 6.5 mg·h/mL. While, an increase in the AAG from 1.11 to 1.85 g/L resulted in roughly a 50 % reduction in the odds of experiencing one toxicity event from the TOX1 group.

Tabl 9. Logistic regression models for adverse events at cycle 1

Endpoint	Predictor	p	Odds Ratio*	(95 % CI)
TOX	AUC (4.2 to 6.5 mg.h/mL)	0.0021	1.81	(1.24 - 2.64)
TOX1	AUC (4.2 to 6.5 mg.h/mL)	0.0005	2.37	(1.46 - 3.87)
	AAG (1.11 to 1.85 g/L)	0.0505	0.47	(0.22 - 1.00)

*odds ratio for covariate change from 25th to 75th percentiles

for AUC and for AAG

Response rate

The overall response rate was 29% in both intent-to-treat and evaluable populations. Baseline AAG was the only significant predictor of response rate (p=0.0039) with an odds ratio of 0.44 for a change in AAG from 1.11 to 1.85 g/L. An increase in the baseline AAG level was associated with a 56% decrease in the odds of response (**Table 10**). The response rate was 41.3% (95% CI : 27.0% - 56.8%) for patients with a low AAG (AAG ≤ 1.11 g/L, n = 46) and 15.9% (95% CI : 6.7% - 30.1%) for patients with a high AAG (AAG ≥ 1.85 g/L, n = 44).

Tabl 10. logistic regression model for response*

Predictor	p	Odds Ratio** (95 % CI)
AAG (1.11 to 1.85 g/L)	0.0039	0.44 (0.25 - 0.77)

* intent-to-treat population, response rate = 25.0 %

*odds ratio for AAG change from 25th to 75th percentiles

In the univariate analyses, in addition to baseline AAG levels, trends were observed for a lower odds of response in patients with metastatic disease (p=0.054), in patients who received radiotherapy prior to docetaxel treatment (p=0.055), in younger patients (p=0.080) and in patients with a poor performance status (p=0.080). However, when baseline AAG was included in the multivariate analysis, none of these covariates entered the model even at a significance level of p<0.10. Similar findings were very obtained for the patients with evaluable disease.

Survival

The most significant univariate predictors of survival were cumulative dose, baseline AAG and number of sites of disease (p<0.0001). Clearance or AUC, prior radiotherapy, gender and performance status were also significant predictors of survival (p<0.05). The risk of death decreased as the cumulative dose of docetaxel increased. However, an increased risk of death was observed for patients with higher AAG, two or more sites of disease, low CL or high AUC, poor performance status, female gender and for pati nts having received prior therapy.

Only cumulative dose, AAG and two or more disease sites remained significant in the multivariate analysis (**Table 11**). Th risk of death decreased by 20 % for each additional cycle of treatment and roughly doubled in patients with a high AAG (1.85 g/L) compared to patients with a low AAG (1.11 g/L) and in patients with two or more sites of disease.

Table 11. Cox regression model for survival*

Predictor	p	Risk Ratio** (95 % CI)
Cumulative dose***(100mg/m²)	< 0.0001	0.82 (0.74 - 0.90)
AAG (1.11 to 1.85 g/L)	< 0.0001	1.76 (1.40 - 2.21)
No disease sites (< 2 to ≥ 2)	0.0049	1.96 (1.23 - 3.12)

* death occurred in 70.5 % of the patients (127 of 180 patients)

** risk ratio for change of covariates given in brackets

*** time-dependent covariate

When baseline AAG was not considered in the stepwise multivariate analysis, it was replaced by performance status (p=0.0053), gender (p=0.025) and prior radiotherapy (p=0.045). Therefore, the pretreatment AAG level appeared to be a more important predictor of survival in NSCLC patients treated with docetaxel than several other known prognostic factors. The median survival (**Table 12** and **Figure 4**) varied from 15.6 months in low AAG patients (AAG ≤ 1.11 g/L, n=46) to 5.5 months in high AAG patients (AAG ≥ 1.85 g/L, n=44). Patients with intermediate AAG values (n=90) had a median survival time of 9.2 months.

Table 12. Survival as a function of alpha-1-acid glycoprotein baseline level

	alpha-1-acid glycoprotein (g/L)			
	≤ 1.11* (n=46)	1.12 - 1.84 (n=90)	≥ 1.85** (n=44)	Log-Rank
median (month)	15.6	9.2	5.5	< 0.0001
95% CI	(11.8-20.0)	(6.4-11.4)	(4.1-7.5)	

* 25% quantile of AAG distribution

** 75% quantile of AAG distribution

Over the last decade performance status has been recognized as the most important predictor of response, and survival in patients with advanced NSCLC (Ginsberg RJ, Vokes EE, Raben A: Non-small cell lung cancer, in De Vita VT, Hellman S, Rosenberg SA (eds): Cancer Principles & Practice of Oncology. Volume 1, Chapter 30, Section 2, 5th Edition, Philadelphia, New York, Lippincott-Raven, 1997, pp 858-911). This study shows that NSCLC patients with a high baseline AAG have a lower response rate (14% compared to 44% in patients with a low AAG) and a markedly shorter survival (median of 5.5 month compared to 15.6 months in patients with a low AAG).

Example 3

This example is representative of methods used to determine AAG levels. 500 μL samples of blood were collected in a polystyrene microtube without anticoagulant by venous puncture. The serum was removed after centrifugation.

For laser nephelometry a Behring Laser Nephelometer module I was used. (Behringwerke, D-3550 Marburg/Lahn, Germany). Samples, standards, and antisera were diluted with sterile isotonic saline solution and 100 µL of 101-fold diluted sample were mixed in a microcuvette with 200 µL of a fivefold diluted anti-orosomucoid antiserum (LN serum anti-orosomucoid (AAG) SAW; Behringwerke). The cuvettes were shaken briefly and allowed to stand for 1 hour at room temperature, and the light scattered by the resulting antigen-antibody complexes was measured (in volts) with the nephelometer. A calibration curve was prepared by use of an 800mg/L standard solution of orosomucoid, diluted to give concentrations of 40, 20, 10, 5, 2.5, and 1.25 mg/L. The blank values (i.e., the light scattered by the empty cuvettes) were negligible (80-150 mV).

Example 4 - This example presents a clinical trial simulation for exploring the safety profile of docetaxel (Taxotere®) in cancer patients.

Docetaxel exposure and alpha-1-acid glycoprotein level (AAG) predict hematological toxicities of docetaxel (Bruno et al., *J. Clin. Oncol.*, 16, 187 (1998)). To assess the impact of increasing doses on the safety profile of docetaxel, in patients with different AAG levels, 100 complete trials were stochastically simulated (ACSL Biomed). In each trial, 600 patients were randomly assigned to groups of either low (L) (≤ 1.11 g/L), intermediate (I) (1.12-1.84 g/L) or high (H) (≥ 1.85 g/L) AAG and received 60, 75, 100 and 125 mg/m^2 of docetaxel intravenously over 1 hour. The simulated median AUC, median incidence of grade 4 neutropenia (GR4) and febrile neutropenia (FEB) were:

Group	Dose (mg/m^2)	60	75	100	125
L	AUC (µg.h/mL)	2.7	3.4	4.5	5.6
	GR4 (%)	68.9	73.4	79.9	84.5
	FEB (%)	4.3	5.3	7.3	10.1

Group	Dos (mg/m^2)	60	75	100	125
	AUC ($\mu g.h/mL$)	3.0	3.8	5.0	6.3
	GR4 (%)	41.1	46.5	56.6	66.3
I	FEB (%)	2.3	2.9	4.4	6.5
	AUC ($\mu g.h/mL$)	3.8	4.7	6.3	7.9
	GR4 (%)	13.3	17.5	26.1	35.7
H	FEB (%)	1.0	1.0	1.9	3.6

The results demonstrate that the dose response of docetaxel is markedly influenced by AAG and these results provide insights for the design of future trials.

Example 5 - This examples summarizes studies on Alpha-1-acid glycoprotein as an independent predictor of efficacy and survival in NSCLC patients treated with docetaxel (Taxotere®).

Baseline alpha-1-acid glycoprotein (AAG) and docetaxel docetaxel clearance (and/or exposure) were previously found to be independent predictors of docetaxel safety (all tumor types combined) and of time to progression (TTP) in NSCLC (Bruno et al., *J. Clin. Oncol.* (Vol. 16, No. 1, 1998, pp. 187-196)). The predictors of treatment outcome and survival of advanced NSCLC patients entered in 4 Phase II studies (n=180) of docetaxel (100 mg/m^2) were investigated using logistic and Cox multivariate regressions. Univariate analysis showed that compared to patients with high AAG ($\geq$ 1.92 g/L (75 percentile)), patients with low AAG ($\leq$ 1.09 g/L (25 percentile)) experienced more side effects (e.g. febrile neutropenia: 19% vs. 2.3%, p=0.02) but had a higher response rate (44% vs. 14%, p=0.002), a longer TTP (18 vs. 9.7 weeks, p=0.006) and a much longer survival: 16 months compared to 5.2 months ($p<0.0001$). In multivariate models, in addition to TTP (Bruno et al., *supra*), AAG was an independent prognostic factor for the incidence of severe side effects at first cycle (p=0.006 with an interaction with clearance), for response rate (odds ratio for non response in high AAG

patients: 5.5, p=0.006), and for survival (p<0.0001). In conclusion, low AAG is independently associated with better efficacy and longer survival in advanced NSCLC treated with docetaxel.

CLAIMS

1. A method for determining the dosage of a taxoid to administer to a patient who is being treated for cancer and whose body fluids include alpha-1-acid glycoprotein comprising: (A) observing the patient's level of alpha-1-acid glycoprotein; (B) evaluating said level to determine the dosage of the taxoid to administer to the patient by comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said evaluation, recommending the dosage of the taxoid to administer to the patient.

2. The method of claim 1 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

3. The method of claim 1 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

4. The method of claim 3 wherein said cancer is non-small cell lung cancer.

5. The method of claim 1 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

6. A method for ass ssing the effect of treatment of a patient who has cancer and who is being treated with a taxoid comprising: (A) observing the patient's alpha-1-acid glycoprotein level; (B) comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a

population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said comparison, assessing the effect of continued treatment of the patient with respect to the patient's response to treatment, the length of survival of the patient, or side effects that may be experienced by the patient.

7. The method of claim 6 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

8. The method of claim 6 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

9. The method of claim 8 wherein said cancer is non-small cell lung cancer.

10. The method of claim 6 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

11. The method of claim 6 wherein said patient is being treated with a dosage of about 55 to about 200 mg/m^2 of taxoid.

12. The method of claim 6 wherein said patient is being treated with about 55 to about 125 mg/m^2 docetaxel.

13. The method of claim 6 wherein said patient is being treated with about 135 to about 175 mg/m^2 paclitaxel.

14. A method for reducing the side effects experienced by a patient who has cancer and who is to be treated with a taxoid comprising: (A) observing the patient's alpha-1-acid glycoprotein (AAG) level; (B) comparing said level to a predetermined alpha-1-acid glycoprotein level derived from a population of patients having said cancer and treated with said taxoid at a common dosage; and (C) based on said comparison recommending the dosage of said taxoid to administer to said patient to reduce the incidence or severity of side effects that the patient may experience during treatment with said taxoid.

15. The method of claim 14 wherein said taxoid is selected from the group consisting of docetaxel and paclitaxel.

16. The method of claim 14 wherein said cancer is selected from the group consisting of breast, ovarian, lung, head and neck, gastric, pancreatic, melanomas, and soft tissue sarcomas.

17. The method of claim 16 wherein said cancer is non-small cell lung cancer.

18. The method of claim 14 wherein said cancer is non-small cell lung cancer and said taxoid is docetaxel.

19. The method of claim 14 wherein said population of patients is being treated with a dosage of about 55 to about 200 mg/m^2 of said taxoid.

20. The method of claim 14 wherein said patient is being treated with about 55 to about 125 mg/m^2 of docetaxel.

21. The method of claim 14 wherein said patient is being treated with about 135 to about 175 mg/m^2 paclitaxel.

22. The method of claim 14 wherein the side effects are selected from the group consisting of neutropenia, infection, diarrhea, infusion-related hypersensitivity reactions, allopenia, neurotoxicity, mucositis, stomatitis, severe asthenia, fluid retention and myalgias.

23. The method of claim 22 wherein said side effect is neutropenia.

24. The method of claim 23 wherein said neutropenia is febrile neutropenia.

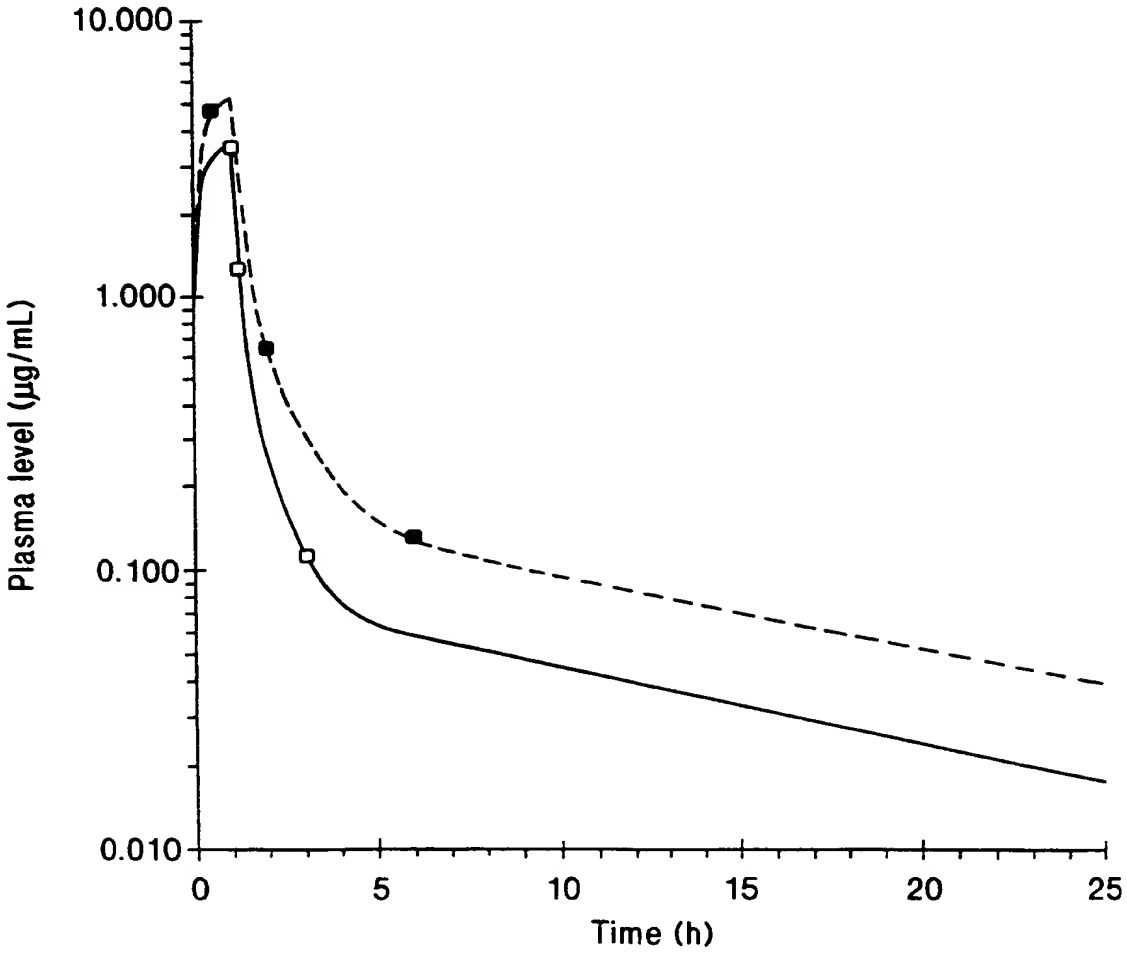
25. The method of claim 14 wherein said taxoid is docetaxel and said dosage is recommended to be less than about 100 mg/m^2.

26. The method of claim 14 wherein said taxoid is paclitaxel and said dosage is recommended to be less than about 175 mg/m^2.

27. The method of claim 14 wherein the recommend d dosage is about 5 to about 35% below said common dosage.

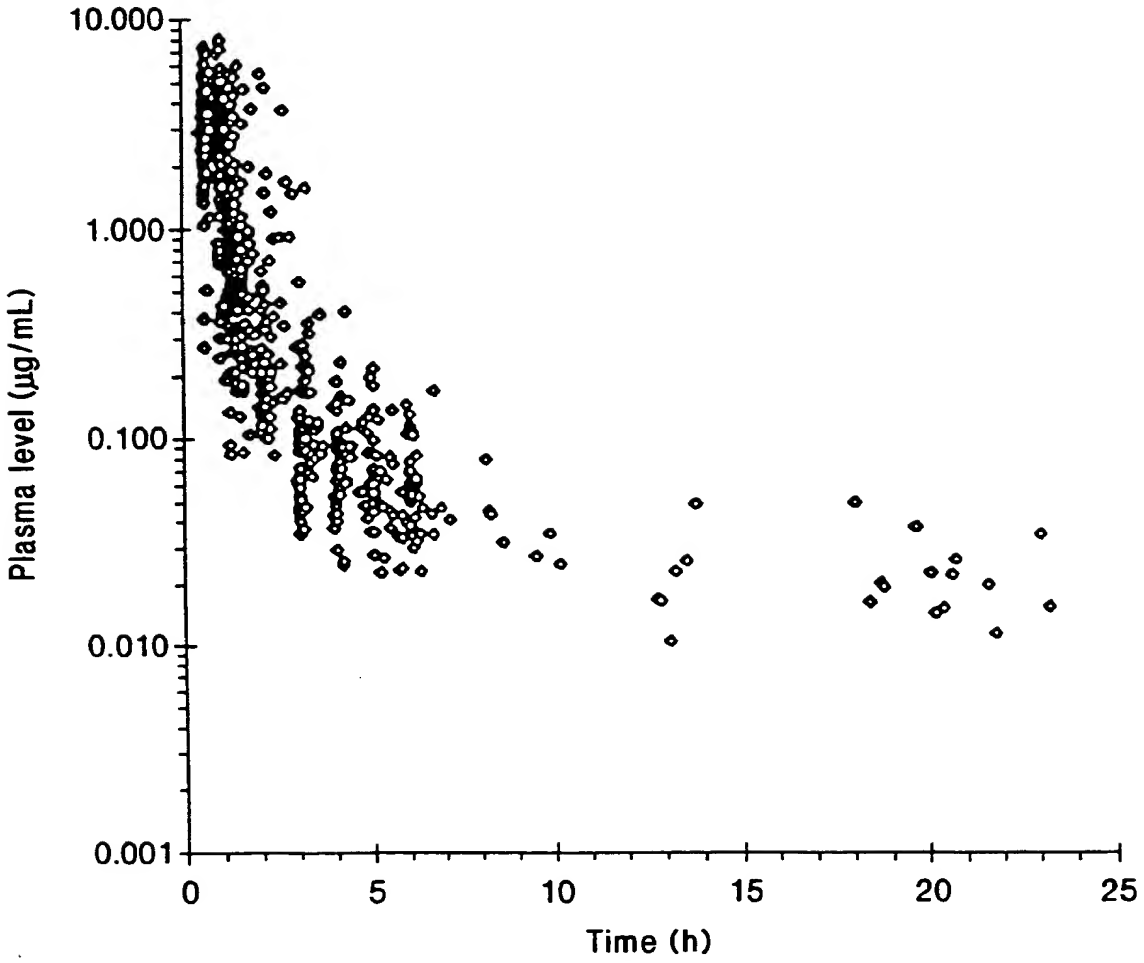
28. The method of claim 14 wherein the recommended dosage is reduced by about 10 to about 30% below said common dosage.

29. The method of claim 14 wherein the recommended dosage is reduced to about 15 to about 27% below said common dosage.



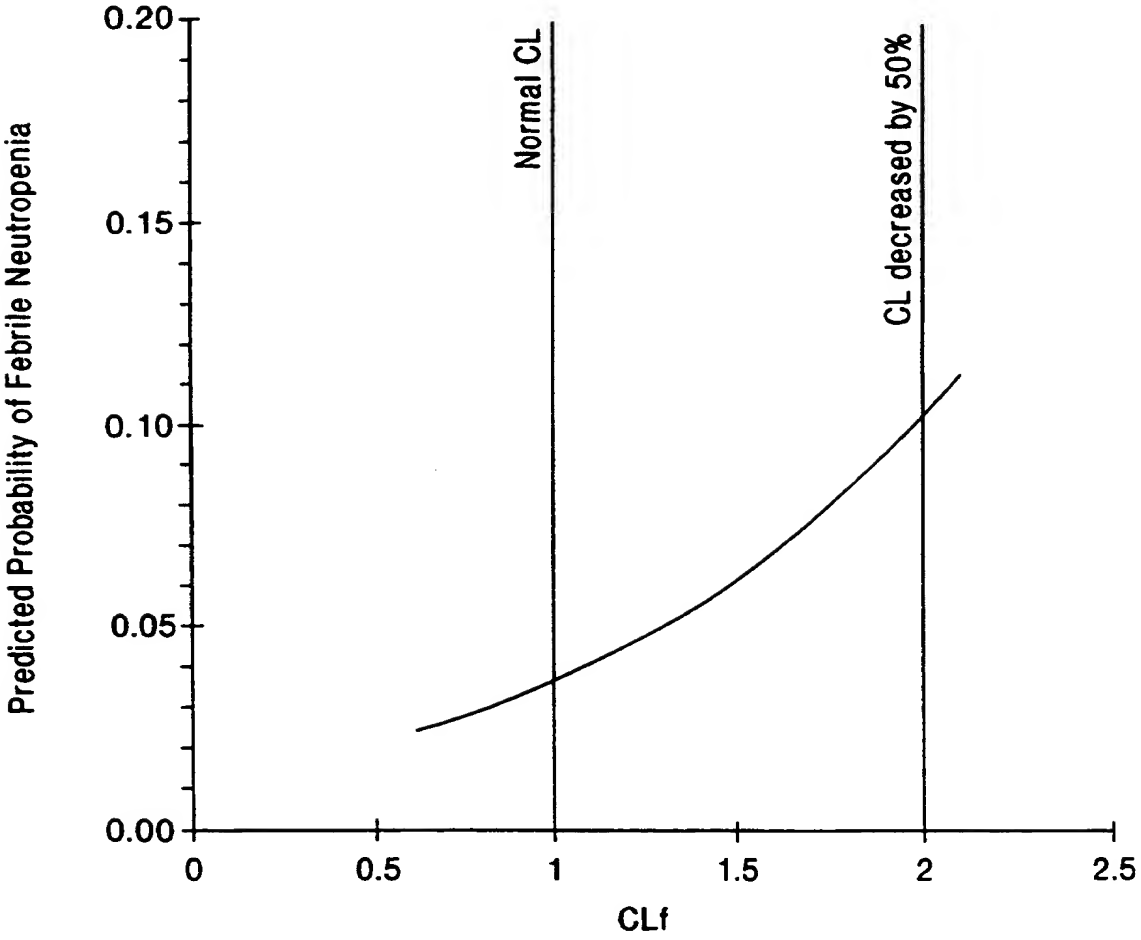
Docetaxel PK profile in representative patient with normal liver function (□) and patient with elevated hepatic enzymes (---●---). Lines denote model predictions after Bayesian estimation.

FIG. 1



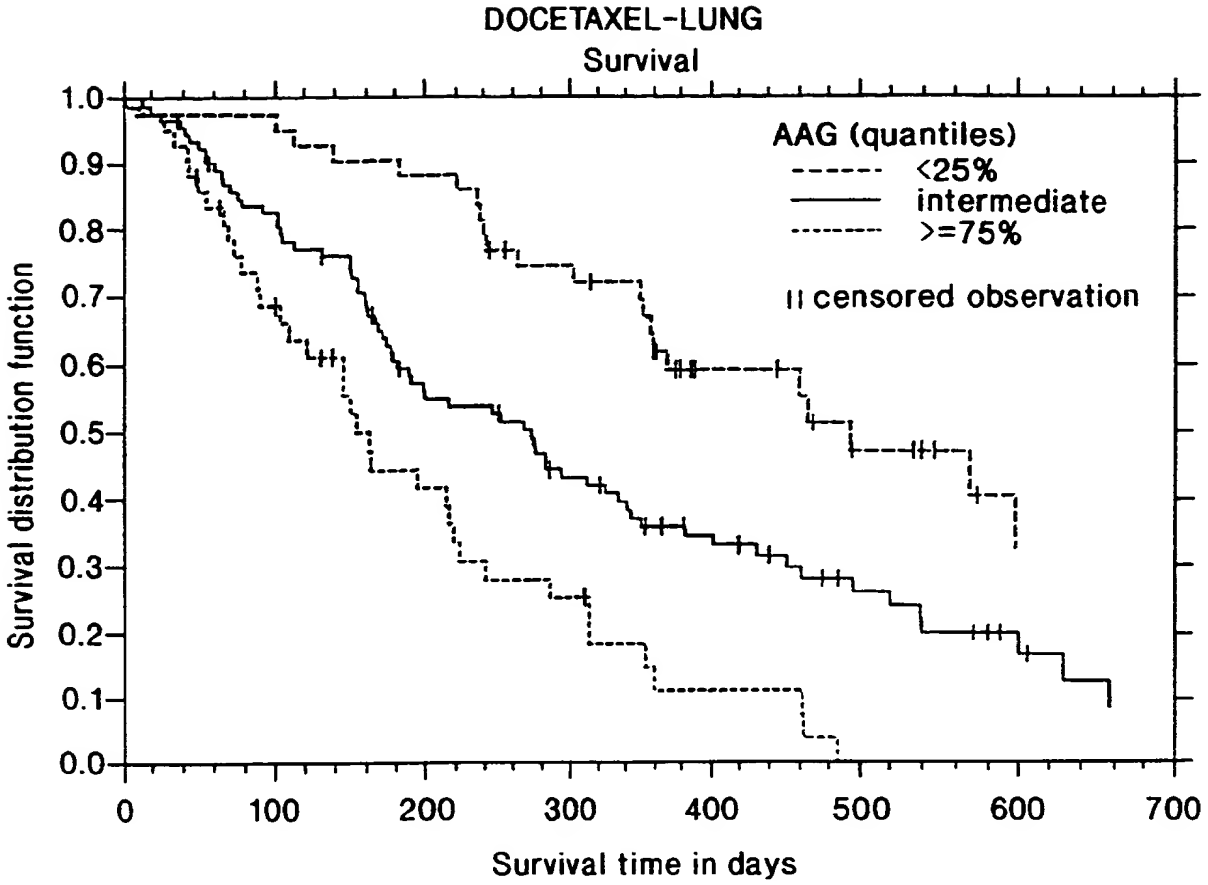
Docetaxel population PK profile in a subset of 254 patients.

FIG. 2



Model-predicted probability of febrile neutropenia as a function of CLf for a patient with median AAG. Reference vertical lines denote normal CL (CLf = 1) and 50% reduced CL (CLf = 2).

FIG. 3



survival curves in NSCLC patients with low (≤1.11 g/L, ——), intermediate (1.12 to 1.84 g/L, ----) and high (≥1.85,-•-•-) baseline AAG (/censored observation)

FIG. 4

INTERNATIONAL SEARCH REPORT

Int. .l Application No
PCT/US 99/31284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	VEYRAT-FOLLET C. ET AL.: "Application of clinical trial simulation in exploring the safety profile of docetaxel (D) in cancer patients" CLINICAL PHARMACOLOGY AND THERAPEUTICS, vol. 65, no. 2, February 1999 (1999-02), page 198 XP000908903 abstract ---	1-13
X	URIEN S. ET AL.: "Docetaxel serum protein binding with high affinity to alphal-acid glycoprotein" INVESTIGATIONAL NEW DRUGS, vol. 14, 1996, pages 147-151, XP000908900 abstract page 150, column 1, paragraph 2 -page 151, column 1, paragraph 1 --- -/--	1-13

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2000	29/06/2000

Name and mailing address of the ISA	Authorized officer
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3

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INTERNATIONAL SEARCH REPORT

Int. al Application No
PCT/US 99/31284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BRUNO R. ET AL.: "A population pharmacokinetic model for docetaxel (Taxotere): model building and correlation" JOURNAL OF PHARMACOKINETICS AND BIOPHARMACEUTICS, vol. 24, no. 2, - 1996 pages 153-172, XP000908881 cited in the application abstract page 169, paragraph 5 page 170, paragraph 5 ---	1-13
X	BRUNO R. ET AL.: "Population pharmacokintetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer" J. CLIN. ONCOL., vol. 16, no. 1, January 1998 (1998-01), pages 187-196, XP000910385 cited in the application abstract page 191, column 1, line 18-20 ---	1-13
X	GANZ P.A. ET AL.: "Monitoring the therapy of lung cancer with alpha-1-acid glycoprotein" CANCER RESEARCH, vol. 44, 1984, pages 5415-5421, XP000909045 abstract page 5416, column 1, paragraph 3 -page 5416, column 2, paragraph 1 ---	1-13
X	GANZ P.A. ET AL.: "Evaluation of a radioimmunoassay for alpha-1-acid glycoprotein to monitor therapy of cancer patients" JOURNAL NATIONAL CANCER INSTITUTE (JNCI), vol. 71, no. 1, 1983, pages 25-30, XP000909046 cited in the application abstract page 26, column 1, paragraph 2 ---	1-13
X	BIENVENU J. ET AL.: "Laser nephelometry of orosomucoid in serum of newborns: reference intervals and relation to bacterial infections" CLIN. CHEM., vol. 27, no. 5, 1981, pages 721-726, XP002139731 cited in the application page 721, column 2, paragraph 5 -page 722, column 1, paragraph 2 --- -/--	1-13

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INTERNATIONAL SEARCH REPORT

Int ·al Application No
PCT/US 99/31284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KREMMER T. ET AL.: "Determination and analysis of human serum alpha-1-acid glycoprotein by liquid chromatographic methods" J. LIQUID CHROMATOGRAPHY, vol. 18, no. 6, 1995, pages 1207-1218, XP000909051 page 1209, paragraph 1 -page 1210, paragraph 2 -----	1-13

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 14-29

Subject-matter for which protection os sought in claims 14-29 is excluded from patentability because it relates to a method for treatment of the human or animal body by therapy (Rule 39.1.(iv) PCT). However, a search has been performed on analytical tests for determining alpha-1-acid glycoprotein levels and on the correlation between alpha-1-acid glycoprotein levels and the pharmacokinetics/pharmacodynamics of taxoids.